











THE JOURNAL  
OF  
EXPERIMENTAL ZOOLOGY

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VOLUME 38  
AUGUST—JANUARY, 1923-24

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY  
PHILADELPHIA, PA.



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## THE EFFECT OF CHEMICALS ON LOCOMOTION IN AMEBA

### I. REACTIONS TO LOCALIZED STIMULATION

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TWELVE FIGURES

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### INTRODUCTION

An understanding of Ameba is commonly considered fundamental for an explanation of the phenomena of life. Among the earlier investigators, Berthold states this very appropriately ('86, p. 1). He says, "Je mehr es der Forschung in den letzten Jahrzehnten gelang, tiefer in die Probleme der biologischen Wissenschaften einzudringen, um so klarer hat es sich gezeigt, dass fast alle in letzter Instanz auf Protoplasmaprobleme zurückführen. . . . Mit der einfachst organisirten Amöba und ihren Lebenserscheinungen sind alle fundamentalen Probleme des Lebens schon gegeben und ein tieferes Eindringen in die

allgemeine Lebensmechanik eines höheren Organismus ist nicht möglich, so lang uns die der Amöba noch unverständlich ist."

The fact that many incompatible theories are still held as to the nature and cause of the activities of *Ameba* shows the great need for more complete knowledge of the objective facts as to this organism. A brief review of such theories will suffice in order to appreciate the situation as it has existed and still exists with regard to *Ameba* and its locomotion. Among the earlier workers Brücke ('62), Schultze ('63), Kühne ('64), and others were of the opinion that protoplasm has some sort of contractile substance, and later Jennings ('04) and Dellinger ('06) adduced experimental data to show more particularly the nature and character of contractile phenomena in *Ameba*. But Berthold ('86) in contrast to the above, was led through the investigations of Quinke and others on liquids, to study in detail phenomena in *Ameba* comparable to those found in liquids and to ascertain the amount of coincidence between them. This method of attack promised much, and as a result of his observations he says (p. 8): "Die Kräfte der Adhäsion und Cohäsion, mit ihrem durch die Umstände bedingten stetigen Wechsel der Intensität sind es also, welche im Protoplasma den Zusammenhang zwischen Stoff und Form, zwischen Stoffwechsel und Formwechsel vermitteln." He held also that the extension and retraction of pseudopods depend upon the composition of the surrounding medium, and that for every change in the surrounding medium there is a corresponding variation in the forces controlling the form and the movement of the amebas. Accordingly he says (p. 102): "Nach der vorstehend entwickelten Auffassung ist also die Ausbreitung des Amöbenkörpers und die Bildung der Pseudopodien kein activer, sondern ein passiver Vorgang, die Pseudopodien werden ausgezogen, nicht ausgestreckt."

The work of Berthold served as a background for another theory of ameboid movement which has become one of those most widely held,—the surface tension theory. The chief exponents of this theory, Bütschli ('92) and Rhumbler ('98, '05), however, do not agree to any large extent with Berthold. They contend that *Ameba* is a complex fluid which moves about as

a result of local changes in surface tension, but does not spread on solids or adhere to them in the way Berthold described. This theory has been held very widely since its formulation and even now has its supporters, although data have steadily accumulated which tend to make it less tenable. For example, Mast and Root in their observations on some feeding reactions in *Ameba*, found by indirect measurements that if the movement in certain processes of feeding is due to a change in surface tension, it requires to perform the work involved, a reduction in surface tension of at least 383 dynes per centimeter. They say ('16, p. 48): "To account for the process [of cutting paramecia in two] on the basis of the surface tension theory, the surface tension of the amebae would have to be, at the very least, much higher than 383 dynes per centimeter and in all probability considerably higher than 1118 dynes per centimeter. The surface tension of protoplasm is, however, only approximately 50 dynes per centimeter. It is therefore probably at best an insignificant factor in the process of feeding in *Ameba*."

Hyman ('17) agrees substantially with Jennings, Dellinger, Mast and Root, and others who hold that the surface tension theory is inadequate to explain ameboid activity. She holds with Rhumbler ('10, '14) that ameboid movement must be due to alterations of the colloidal state, and that liquefaction or solation is the cause of the extension of a pseudopod and coagulation or gelation of the withdrawal of pseudopods and of active contraction. The liquefaction she believes to be brought about by a metabolic change contingent upon a metabolic gradient which arises in the ameba before the pseudopod appears.

Schaeffer ('20, p. 7) restates his earlier contention that the mechanism controlling locomotion and feeding is internal and that Rhumbler's statement to the effect that changes in behavior are directly deducible from the action of stimuli in effecting liquefaction or gelation of the ectoplasm does not hold in many cases of feeding. He maintains that ectoplasm is continually formed from endoplasm at the anterior end and the reverse at the posterior end and he says (p. 142): "One of these special

aspects of streaming in amebas is the formation of ectoplasm. For ectoplasm formation is not essential to streaming. But it is almost certainly essential to locomotion, for locomotion has not been observed in amebas where ectoplasm was not formed. But, on the other hand, ectoplasm, as known in the amebas, is not formed without streaming. . . . Streaming is therefore the fundamental process in ameboid locomotion."

He also postulates a third layer which he contends (p. 74) from "all the evidence available, both direct and indirect, points to the conclusion that the behavior of the surface layer on the ameba resembles in general and in detail the behavior of the surface tension layer in an inert drop of fluid, and that we must regard the surface layer on the ameba as a true surface tension layer. This layer is therefore a dynamic layer, containing free energy, and capable of performing work. It is physiologically distinct from ectoplasm as ectoplasm is distinct physiologically from endoplasm. . . ." He says further (p. 142): "From the point of view of ameboid movement, the discovery of the surface film narrows down the problem very considerably . . . it shows clearly that the regions where ectoplasm is most rapidly formed is also the region where the superficial tension is increased."

From this brief review it is clear that no theory as yet advanced has received general acceptance as accounting for the activities of Ameba. Further knowledge of the concrete facts is required. The writer, therefore, undertook this investigation primarily with a view to determining precisely what reactions result from local changes in the surface of Ameba induced by chemicals under strictly localized conditions in a medium whose constitution is accurately known. Such experimentation should reveal something concerning the nature of the local changes at the surface and the probable rôle played by these in the phenomenon of ameboid movement. In a second paper, data will be set forth concerning the effect on movement and other processes of total immersion in various solutions.

This investigation was undertaken at the suggestion of Prof. S. O. Mast, under whose direction its outline was made, the

instrument used in applying the various chemicals perfected, and the manuscript prepared. The writer thanks Professor Mast very heartily for his assistance. He is likewise indebted to Professor Jennings for friendly criticism in revising the manuscript.

Grateful acknowledgment is hereby made also for the assistance in the preparation of solutions and in many other ways to Dr. J. Fitch King, Mr. Charles E. Bills and Dr. Leslie C. Beard. Their extensive knowledge of chemistry has been available to him at all times during the investigation.

#### MATERIAL AND METHODS

The amebas used in the following experiments correspond in every way with Schaeffer's ('20, p. 37) description of *Ameba proteus*, except that the nucleus is biconcave. They were obtained originally from a stream in the vicinity of Baltimore and transferred to cultures previously prepared as follows: Distilled water, 100 cc., and finely chopped raw timothy hay 0.25 to 0.5 gm., were put into flat finger-bowls 10 cm. in diameter and 5 cm. deep and allowed to stand for two or three days, after which it was inoculated with amebas. In other instances, if the hay and water solution seemed too strong, it was again diluted by one-half or one-third, as the situation seemed to warrant. At the end of every four or more weeks, about half the culture fluid was siphoned off and fresh hay and water added in the proportions stated above. In this way, amebas were kept in a flourishing condition for more than two years.

For each experiment, the amebas with a few drops of culture fluid were taken from a culture dish and put for ten minutes or less into 100 cc. distilled water and then transferred to a second and later to a third dish containing 10 cc. distilled water, where they were left for five minutes in each instance. They were then carefully aggregated at the center of the dish by using a fine glass needle and removed in a capillary pipette with the least possible amount of water and placed in a small watch-glass containing N, 500 KCl, after which various chemicals

were applied locally as indicated below. In some experiments they were not put into KCl. Wherever this occurred it is so stated in the experiment. In KCl, N/500, for 8 or more hours, *Ameba* travels continuously and preserves a monopodal form. Neither this salt nor this concentration is the only one in which *Ameba* travels continuously and monopodally, but this salt was first found to be satisfactory and hence was used. The necessity for using any salt solution arose from the fact that when allowed to remain in distilled water they do not travel appreciably and their form is not suitable for local application. The writer believes he has been enabled to obtain more accurate observations by using the above salt since the form of *Ameba* in it is consistently monopodal, and therefore any change in the direction of locomotion through the extension of pseudopods or otherwise can be more carefully noted.

All observations were made under a Leitz binocular microscope, to which was attached a device similar in principle to the Barber pipette-holder (fig. 1).

Local stimulation was effected by bringing the tip of a capillary pipette containing the chemical desired close to but not in contact with the *ameba* and allowing the chemical to diffuse therefrom. For the details of the manner in which the pipette was held in position and made manipulable see figure 1.

The pipettes used in the experiments were of Jena glass. The size of the lumen at the tip (usually about 15 to 20 $\mu$  in diameter) was ascertained by putting the tip under water and observing the size of bubbles produced. After some practice, pipettes were readily made with apertures which covered only a very small portion of the surface of an *ameba*. The pipette used was filled with the solution desired by inserting into it a second slender pipette and ejecting the solution, and then after removing the second pipette, forcing the solution to the tip by means of a strong rubber bulb attached to its base.

A control pipette was employed from time to time during the experiments. This contained in each experiment a solution identical with that in which the *amebas* were when the chemical tested was applied.



Fig. 1 Photograph of mechanism for holding the capillary used in the local application of chemicals to Ameba.

The capillary portion of the pipette was broken off, the pipette washed and redrawn after using each reagent or a different concentration of the same reagent.

The chemicals used were in all cases the purest available. These consisted of certain acids, hydroxides, salts, alkaloids and non-electrolytes.

## OBSERVATIONS AND RESULTS

### 1. *Acids*

The effect of hydrochloric, nitric, sulphuric, hydrocyanic, oxalic, and carbonic acids was ascertained. Of these, hydrochloric and oxalic were used most extensively and in concentrations of N/1, N/5, N/500, N/1000, N/2000, N/3000, N/4000, N/5000, N/10,000, N/15,000, N/20,000, N/30,000. Hydrocyanic was also used extensively, but in a more limited range of concentration (N/100 to N/500).

The usual method of procedure with any given reagent was to allow it to diffuse from a distance of about  $40\mu$  against the surface of the ameba at two or three different points at the anterior end, along the sides, and at the posterior end.

The response to acids in general consists of a local movement of the protoplasm toward the pipette in the form of a projection. This projection varies from a small protuberance formed immediately and very eruptively in high concentrations (N/1 to N/500), to a small and slender one formed gradually in low concentrations. The following descriptions with diagrams of the reactions which are in a general way typical of all the acids used, will illustrate their obvious character. In the application of HCl, N/1, to the advancing anterior tip of an ameba, there is almost immediately an eruptive forward flow followed by cessation and reversal of streaming. A pseudopod<sup>1</sup> then forms either at the posterior end or laterally (fig. 2; A, 1 and 2). When the stimulus is given laterally, the positive response, consisting in the formation of a protuberance at the point stimulated, is

<sup>1</sup> The use of the word pseudopod to indicate the protuberances formed when chemicals are applied locally to *Ameba*, signifies nothing concerning a possible comparison between such protuberances and the real pseudopods that are formed in normal locomotion.

quickly followed (usually) by the extension of a pseudopod directly opposite the region stimulated (fig. 2; A, 3).

In concentrations of  $N/500$ ,  $N/1000$ ,  $N/1500$ , etc., the response consists in the formation of a small and slender pseudopod which, however, rarely persists. Frequently the initial positive response is followed within a few seconds by a branching of the pseudopod which was formed in response to stimulation. These branches extend on either side of the pipette and curve toward it (fig. 2; B, 1). In these lower concentrations the response is

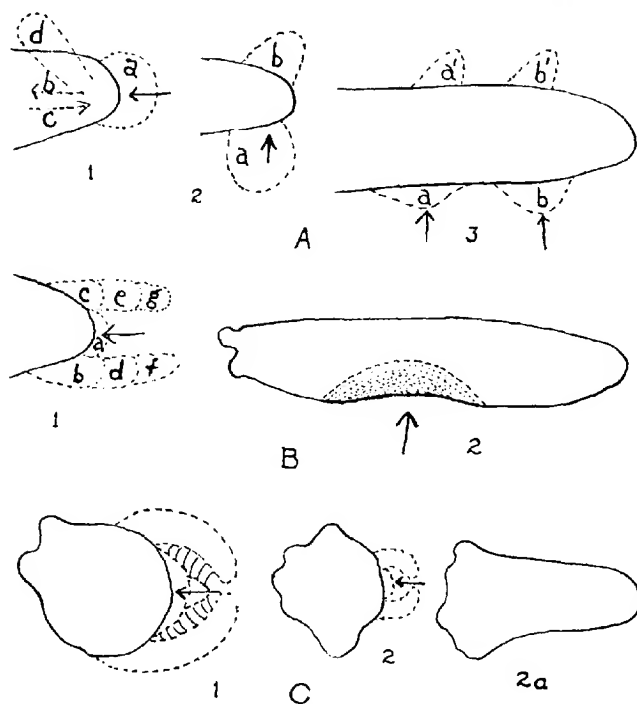


Fig. 2 Sketches illustrating reactions of Ameba to local application of HCl. Solid arrows, point stimulated; broken arrows, direction of protoplasmic streaming; broken lines a, b, c, etc., successive outlines of protuberances formed in reaction. A,  $N/1$  HCl with Ameba in  $N/500$  KCl. B,  $N/2000$  HCl with Ameba in  $N/500$  KCl. C,  $N/10,000$  HCl with Ameba in  $N/4000$  KOH.

not so immediate or so eruptive as in the higher ones. In a few instances when  $N/20,000$  HCl was applied at various points over the surface of the anterior half the pseudopod initiated persisted. If it is applied about half-way between anterior and posterior ends, frequently an overarching shelf of protoplasm is formed, the arch being highest at the point directly in front of the pipette (fig. 2; B, 2). This soon disappears as locomotion continues. If the pipette is allowed to remain at the anterior end of an advancing pseudopod, the branches which arise and extend on either side of the pipette are occasionally further supplemented by delicate hyaline pseudopods which arise at the base of the two branches and extend upward and forward and downward becoming united with each other and with the first two branches by means of a sheet of ectoplasm which flows forward from between them. In concentrations lower than  $N/20,000$  the response is very slight if there is any.

*Influence of medium.* When HCl,  $N/6000$  or  $N/10,000$  is applied to specimens in NaCl,  $N/500$ , instead of KCl,  $N/500$ , the response, if the acid is applied within the first two or more hours, consists in the extension of a finger-like protuberance toward the capillary. Some of these protuberances persist for a minute or more and resemble in their formation normal pseudopods. This is conditioned by the extreme dilution of the acid as well as the sodium chloride medium in which the amebas are when the acid is applied. The surface seems to be continuously modified favorable to the further extension of the protuberance by the application of the acid when the application does not exceed 50 seconds. After 50 seconds or less the reverse of the above process occurs and the protuberance is withdrawn even though diffusion of the acid continue. Amebas that have been immersed in NaCl,  $N/500$ , for 18 or more hours respond to the above concentrations of acid by a contraction of the ectoplasm at the point of application and the extension of a protuberance away from the diffusing acid.

When the above concentrations of acid are applied to specimens in KOH,  $N/4000$ , a slight protuberance is formed at the point of application followed by the extension of one or more

pseudopods on either side of the pipette. These curve toward the tip of the pipette and a third pseudopod half-way between the first two arises and advances directly toward the tip of the pipette. The tips of the 3 pseudopods meet and a thin sheet of ectoplasm flows forward from their bases forming a protoplasmic cup (fig. 2; C, 1 and 2). Vibration of the pipette serves to widen and extend the bay formed by these pseudopods. The above happens when the pipette is allowed to remain after the response has been initiated. If it is removed as soon as the response is initiated, the protoplasmic cup is only partly formed and the ameba soon reassumes the spherical or radially lobose form which characterizes it when immersed in a basic solution. The response obtained by applying very dilute acids to amebas in a basic solution is strikingly similar to that observable when some ciliate protozoön comes to rest against the surface of an ameba, i.e., a bay is formed in which the protozoön is subsequently often caught by the coming together of the tips of the encircling pseudopods and the advance of an overarching wave of ectoplasm, from their bases. Inactive specimens when stimulated for only a few seconds usually put forth slight pseudopods on either side of the pipette. These do not unite to form the protoplasmic cup which is formed if the pipette is allowed to remain for a longer period, but unite in a common pseudopod which extends to considerable length and is then withdrawn (fig. 2; C, 2a).

If the acid is applied half-way between the anterior and posterior ends of such specimens as remain extended and are very slowly active, a convex overarching shelf of protoplasm is formed in the region stimulated and the two ends are drawn together somewhat so that the ameba appears crescent-shaped.

## 2. Bases

The effects of sodium, potassium, and ammonium hydroxide were ascertained. Of these sodium and potassium do not differ in effect in any important respect. If N 1. NaOH or KOH is applied at any point on the surface of Ameba there is an immediate rupture of the ectoplasm at that point (fig. 3; A, 1a).

through which the endoplasm flows until nearly all of it has passed into the surrounding medium. Nothing remains except a few crystals which adhere to the inner surface of the ectoplasmic sheath. The pipette was frequently not allowed to remain after the rupture of the ectoplasm, but in no instance did cytolysis stop unless the specimen was quickly moved to a different part of the dish after the reagent was applied and then it stopped only occasionally. After the nucleus had been ejected, cytolysis more frequently continued until complete; but, if the rupture temporarily closed, the specimen underwent intermittently a slow cytolysis during which a few granules were discharged. If cytolysis was stopped before the nucleus was discharged, the specimen soon became normal. The ruptured place could at any time be closed by applying acid either normal or more dilute without moving the specimen from the position in which the NaOH had been applied. In figure 3; A, 1b and 1c is shown the coagulated mass of endoplasm caused by applying quickly N/1, HCl and the immediate closure of the ruptured place.

When N/5 KOH or NaOH is applied at the anterior end of an ameba in N/500 KCl, there is an immediate momentary cessation in streaming, then the protoplasm flows rapidly forward dividing into small lobes after which streaming stops for a moment and is diverted into a recently initiated lateral pseudopod (fig. 3; A, 2). This pseudopod persists and streaming continues obliquely forward, or at right angles to the original direction of locomotion. When the stimulus is applied laterally, the response is slightly delayed as compared with that given at the anterior end. The ectoplasm flows forward in a broad hyaline wave extending almost from anterior to posterior ends; then the endoplasm flows into this clear area in the form of

Fig. 3 Sketches illustrating reactions of Ameba to local application of KOH and NaOH. Symbols same as in figure 2.

A, 1a, 1b, 1c, N/1 NaOH with Ameba in KCl N/500 or culture fluid.

A, 2, 3, N/5 NaOH with Ameba in KCl N/500.

B, N/100, N/500, N/1000 KOH with Ameba in KCl, N/500.

C, N/2000 KOH with Ameba in culture fluid.

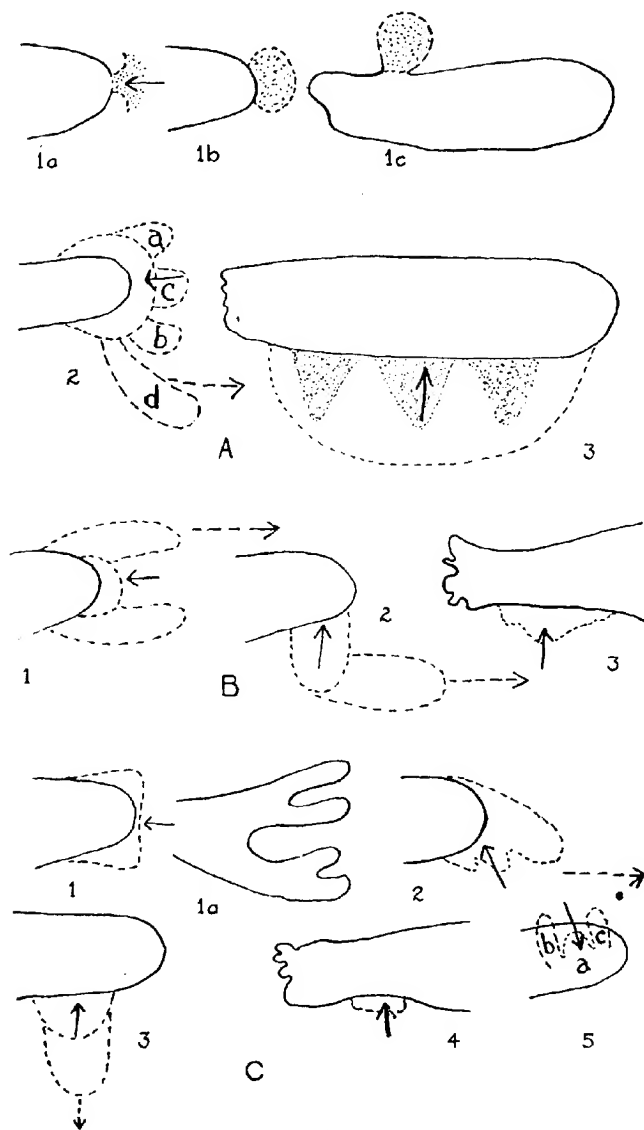


Fig. 3

three pyramidal cones (fig. 3; A, 3). The original position of the endoplasm remains unchanged for 15 to 20 seconds, and since streaming usually is resumed in the original direction or in one opposite to that from which the reagent is applied, the main body of the endoplasm remains in its first position. After this sheet of ectoplasm forms there is no further response at this point.

The ectoplasm tends to stay for some time considerably separated from the endoplasmic inclusions in the stimulated region. When the stimulus is applied near the posterior end, the response consists of a small protuberance and the direction of locomotion is but slightly affected, there is no appreciable response when the stimulus is applied directly at the posterior end. When the reagent is applied at the anterior end in weaker concentrations (N/100, N/500, N/1000) streaming continues forward a few seconds, followed by a cessation of streaming and of ectoplasmic advance at the point stimulated, but streaming continuing from the posterior end, causes the endoplasm to accumulate at the anterior end and to distend it. A small lateral pseudopod arises alternately or simultaneously on either side of the bulb-like anterior end into one of which streaming is continued (fig. 3; B, 1). If the active branch of a slightly dipodal amoeba is stimulated in the same way as a monopodal one, the initial positive response is followed by a reversal of streaming into the inactive branch; or, in case streaming is active in both branches, there is a slight acceleration of streaming in the stimulated branch after which this branch is withdrawn and streaming continues in the other branch.

Lateral stimulation induces near the anterior end the extension of a blunt, short pseudopod. Usually another pseudopod arises at right angles to this one and parallel to the first direction of locomotion (fig. 3; B, 2). The response consists in a slight ectoplasmic advance when the stimulus is applied along the posterior lateral half, but it has no apparent effect on either rate or direction of locomotion and the protrusion formed soon disappears (fig. 3, B, 3). Here again the response is greatest at the anterior end and decreases progressively the nearer the posterior end is approached.

*Influence of medium.* If the amebas are in culture fluid diluted one-half with distilled water in place of KCl, N/500, and N/2000 KOH is applied directly in front of the advancing pseudopod, its tip enlarges into a bulb-like shape after which a concavity arises and two pseudopods are extended on either side of the pipette. These two again fork so that four or more small pseudopods arise (fig. 3; C, 1, 1a). Streaming continues in one of these, usually in one of the last to be initiated, while the others are withdrawn. When stimulated immediately adjacent to the anterior end and the capillary is removed as soon as the response has begun, a pseudopod is extended which frequently persists and determines for a time the subsequent direction of locomotion (fig. 3; C, 2 and 3). If the pipette remains, the pseudopod which is put forth branches and in one of these branches streaming is continued. Stimulation near the posterior end is productive of only a very small protuberance (fig. 3; C, 4).

If the pipette is lowered from above over the anterior portion of the ameba, the response consists in the formation of several slender pseudopods which extend directly and freely up into the water toward the capillary. In one of these, streaming continues until the pseudopod has attained a certain length, then it curves downward and becomes attached to the substratum (fig. 3; C, 5).

If to the anterior end or the sides of amebas in N 10,000 HCl, N 4000 KOH is applied, streaming is directed in an opposite direction from the capillary through the formation of a pseudopod on the side directly opposite the region stimulated.

Ammonium hydroxide differs markedly in its effect from the hydroxide of either sodium or potassium. When a normal solution of it is applied to specimens in culture fluid, the initial response occurs within two to three seconds and consists in a momentary complete cessation of streaming followed immediately by a violent movement forward after which movement and streaming stop and the specimen assumes a pear-shaped appearance (fig. 4, 1b). In a few experiments, a conspicuous hyaline area appeared at the anterior tip. This persisted for five or more minutes during which regular contractions and expan-

sions occurred from posterior to anterior end, but the line between the hyaline area and the endoplasmic granules behind it remained constant (fig. 4, 1c). The ectoplasmic area during these expansions and contractions was shifted from side to side and up and down as indicated in figure 4, 1c, b, b, a, a. The ectoplasmic membrane collapsed and distended with each change of the hyaline portion as it oscillated from side to side and up and down, i.e., the contour of the anterior tip was for an instant smooth as if stretched and for an instant collapsed and wrinkled. Slight eddies occurred at the outer fringe of the endoplasmic granules as this clear area was shifted from side to side and up and down. This phenomenon was manifested for five or more minutes after which the endoplasmic granules gradually filled up the clear region and simultaneously a clear area was seen around the posterior end. It appeared that there was a homogeneous solution of some kind enveloping the endoplasm so that there was seen a distinct hyaline area of increasing width from the anterior to the posterior end where it was most conspicuous (fig. 4; 1d). This area gradually disappeared as the ameba lost its blunt, club-shaped appearance and became more elongated and flattened. Either the endoplasmic constituents become more evenly distributed throughout the hyaline area or there is loss of fluid from the cell with corresponding loss in turgidity, so that the ectoplasm is hardly to be distinguished from the endoplasm. Within twenty minutes, locomotion is slowly resumed.

Lateral stimulation along the anterior half has much the same effect as it has at the anterior end except that no hyaline area develops.

When N/100,  $\text{NH}_4\text{OH}$  is applied at the anterior end, several eruptive advances occur, after which streaming is reversed and a pseudopod is formed laterally. In the case of lateral stimulation the response is positive and eruptive followed by the formation of a pseudopod on the side opposite. If stimulated for one second the positive response is very slight, followed by a momentary pause in streaming but no change in the original direction of locomotion.

When concentrations of N/500, N/1000, N/2000 are used, there is no appreciable response.

### 3. Salts

(1) *Ammonium chloride, sulphate and carbonate.* There is a momentary pause when  $\text{NH}_4\text{Cl}$ , N/1, is applied at the anterior end of specimens in N/500 KCl, then the streaming continues toward the pipette. If the pipette is gradually moved in various directions, the advancing pseudopod also changes correspondingly (fig. 5; A, 1, 2). Lateral application of this reagent induces

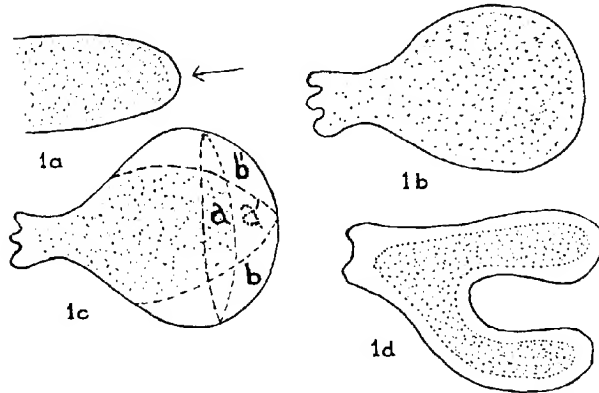


Fig. 4 Sketches of a single specimen illustrating the reaction of Ameba to N/1  $\text{NH}_4\text{OH}$ . Symbols as in figure 2. For full explanation see text.

the formation of a pseudopod which does not persist if the reagent is allowed to diffuse for only 5 to 20 seconds, but does persist if it diffuses for longer periods (25 to 50 seconds) (fig. 5; A, 3). This is true when the reagent is applied at any point on its surface from the anterior to within a short distance of the posterior end. There is no appreciable response when the reagent is applied directly at the posterior end save in some instances when the pipette is kept continuously almost in contact with the posterior end for several minutes. Then within  $1\frac{1}{2}$  minutes streaming becomes slower; stops completely in about

two minutes; and at the end of three minutes the posterior end bulges outward at a spot slightly eccentric to the point of application. If the pipette is now removed the original anterior end, in about a minute, becomes contracted and the bulge assumes the appearance of a newly formed pseudopod. As the new pseudopod is extended the original posterior end (seen like an excrescence) approaches nearer and nearer the original anterior tip which it reaches in about two minutes, after which the direction of locomotion is completely reversed (fig. 5; A, 4 a, b, c, d).

When  $N/100$  or  $N/200$   $\text{NH}_4\text{Cl}$  is applied to specimens in either  $N/500$   $\text{KCl}$  as above, or in culture fluid diluted 50 per cent with distilled water, a pseudopod is extended directly toward the pipette. This usually persists. If these concentrations are also applied to specimens in  $\text{NaCl}$ ,  $N/500$ , a pseudopod is extended from a point on the side directly opposite to that at which the reagent was applied (fig. 5; B, 5, 6). This type of response increases in definiteness the longer the specimens have been in the  $\text{NaCl}$  solution.

When  $(\text{NH}_4)_2\text{SO}_4$ ,  $N/1$ , is applied locally to *Ameba* in  $\text{KCl}$ ,  $N/500$ , the response is similar to that obtained in the application of  $\text{NH}_4\text{Cl}$  to specimens in  $\text{KCl}$ ,  $N/500$ , but when  $(\text{NH}_4)_2\text{SO}_4$  is applied in molar concentration, at the anterior end or along the sides there is a violently eruptive advance of the protoplasm toward the pipette followed immediately by a reversal of streaming and then several consecutive reversals so that the protoplasm seems to be churned up now at one point, now another. Within a few minutes streaming stops altogether. When the reagent is applied at the posterior end a small pseudopod forms which occasionally persists.

The response to  $\text{NH}_4\text{HCO}_3$ ,  $N/1$ , consists in the extension of a pseudopod directly opposite the area to which the reagent is applied (fig. 5; C, 7, 8).

(2) *Sodium chloride and a mixture of equal parts of  $N/100$  sodium nitrate, sodium sulphate, and sodium acetate.* When  $\text{NaCl}$ ,  $N/1$  or  $N/100$ , is applied to the anterior end or laterally the response in some instances consists in the extension of a

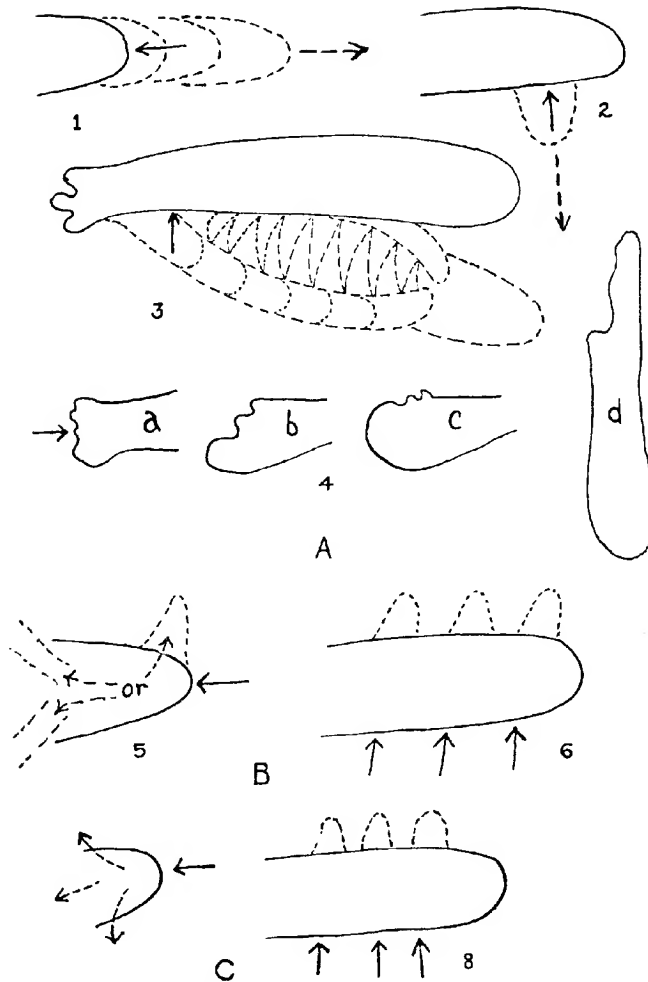


Fig. 5 Sketches illustrating reactions of Ameba to local application of  $\text{NH}_4\text{Cl}$  and  $\text{NH}_4\text{HCO}_3$ . Symbols as in figure 2.

A, 1 to 5, N/1  $\text{NH}_4\text{Cl}$  with Ameba in KCl, N/500.

B, 5 and 6, N/1  $\text{NH}_4\text{Cl}$  with Ameba in NaCl, N/500.

C, 7, 8, N/1  $\text{NH}_4\text{HCO}_3$  with Ameba in KCl, N/500.

pseudopod in a direction opposite to that in which the reagent is applied (fig. 6; A, 1, 2). In others when the reagent is applied at the anterior end the endoplasm accumulates there so that the tip appears bulbous. Streaming is then resumed by the extension of a pseudopod to one side of the pipette (fig. 6; B, 1), but parallel to the first direction of locomotion. When the reagent is applied laterally, the area stimulated contracts so that within a few seconds almost the entire lateral surface becomes the concave side of a crescent (fig. 6; B, 2). When the reagent is applied nearer the anterior end, the region of curvature does not extend so far as to affect the posterior half so that the specimen appears hook-shaped at the anterior end. The response to NaCl, N/150, is essentially similar to that obtained with N/100 when the former is applied to specimens in KCl, N/500, but differs somewhat for specimens in culture fluid. In the latter instance when the reagent is applied at the anterior end there is a momentary pause in ectoplasmic advance and a slight accumulation of endoplasm there; after which a pseudopod is initiated which frequently persists or, the original pseudopod may branch into two or more smaller pseudopods, in one of which streaming is continued (fig. 6; B, 3). When the reagent is applied laterally there is a momentary bulge at the point of application and then streaming is reversed, after which two or more small pseudopods arise on either side of the stimulated place and streaming is again reversed and two or more pseudopods arise at the anterior end (fig. 6; B, 4 a, a, b, c). There is then no further response. If the reagent is applied for twice as long a time as above, four or more pseudopods arise and the response is then the same as in the above.

A mixture containing equal parts of sodium nitrate-sulphate-acetate (N/100 and N/400) was applied to specimens in culture fluid. The response with each concentration consisted in the extension of a small pseudopod toward the pipette at the point of application.

(3) *Potassium chloride and nitrate.* When amebas are in KCl, N/500, and there is applied KCl, N/1 or N/100, a slight contraction of the ectoplasm occurs at the point of application

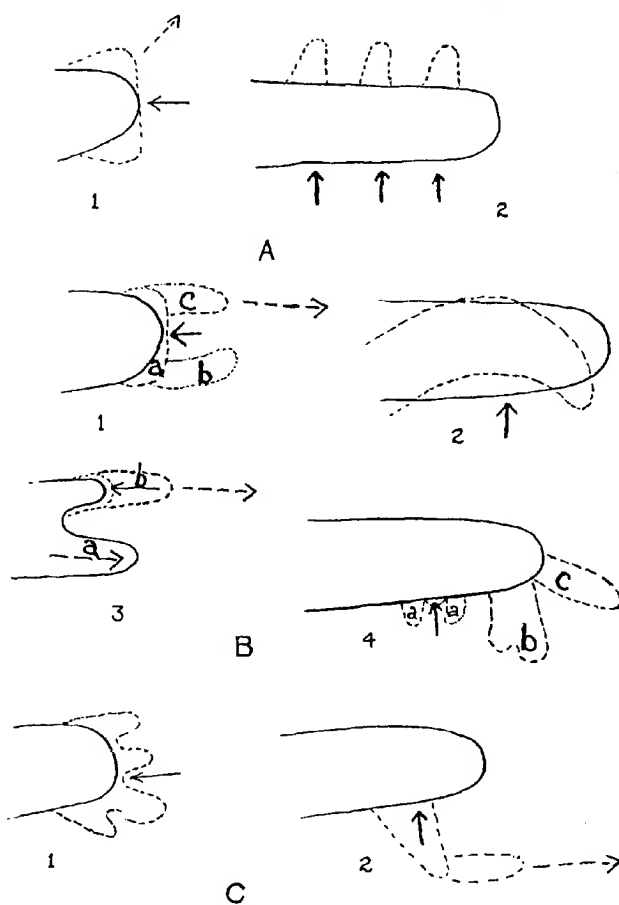


Fig. 6 Sketches illustrating reactions of Ameba to local applications of NaCl and BaCl<sub>2</sub>. Symbols as in figure 2.

A, N/1 NaCl with Ameba in KCl, N/500.

B, 1, 2, NaCl N/100 Ameba in KCl N/500.

B, 3, 4, NaCl, N/100 Ameba in culture fluid.

C, N/100 BaCl<sub>2</sub> Ameba in culture fluid.

and a pseudopod forms on the opposite side. If, however, the amebas are in culture fluid, and KCl in the above-mentioned concentrations is applied, there is a small pseudopod put forth, but this persists for only a short time.

If  $\text{KNO}_3$ , N/300, is applied to specimens in HCl, N/10,000, a pseudopod is formed at right angles to the source of application, on either side if the reagent is applied at the anterior end, and on the opposite side if the reagent is applied laterally.

(4) *Rubidium nitrate*. When  $\text{RbNO}_3$ , N/100, is applied locally to specimens in culture fluid a small protuberance arises at the point of application followed very soon by a pseudopod which arises near it. The latter persists and determines the direction of locomotion. When it is applied to specimens in NaCl, N/500, the response consists of a pseudopod formed in a direction away from the region where the reagent was applied.

(5) *Barium, strontium, calcium, and magnesium chloride*. These reagents,  $\text{BaCl}_2$ ,  $\text{SrCl}_2$ ,  $\text{CaCl}_2$ , and  $\text{MgCl}_2$ , N/100, applied to specimens in culture fluid induce the formation of a small protuberance directly where the reagent is applied followed by the extension of several pseudopods adjacent to this protuberance (fig. 6; C, 1 and 2). These frequently curve toward the pipette and fuse together thus forming a protoplasmic cup.

#### 4. Alkaloids

The alkaloid salts, morphine and strychnine sulphate, quinine and papaverine hydrochloride, and the alkaloids, cocaine, strychnine, and quinine were used. Morphine and strychnine sulphate, cocaine and strychnine were used in M/50 concentrations; quinine in M/500 and papaverine hydrochloride in M/100 and M/500. Quinine, quinine hydrochloride, and cocaine were applied to specimens both in culture fluid and in KCl solution; the others only to specimens in KCl.

Morphine and strychnine sulphate when applied at any point on the anterior two-thirds of the surface of *Ameba* cause streaming to be diverted in some other direction and in general in an opposite direction from the pipette (fig. 7; 1b, 2). Papa-

verine hydrochloride causes a cessation in ectoplasmic advance during which the endoplasm piles up behind the region stimulated. Within thirty or more seconds a pseudopod forms in a direction away from the pipette (fig. 7; B, 1, 2). Quinine and

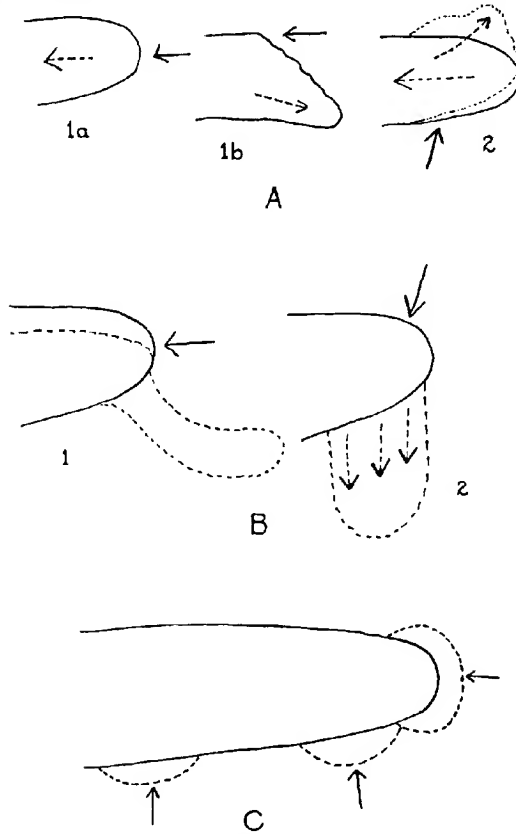


Fig. 7 Sketches illustrating reactions of Ameba to local applications of alkaloids. Symbols as in figure 2.

A, M/50 morphine and strychnine sulphate with Ameba in KCl, N/500 and in culture fluid.

B, M/100 and M/500 papaverine hydrochloride with Ameba in KCl, N/500.

C, M/50 cocaine and M/500 quinine with Ameba in KCl, N/500.

quinine hydrochloride and cocaine cause the protoplasm to flow away from the pipette when they are applied to specimens in culture fluid in a way comparable to that sketched in figure 6; A, etc. Quinine and cocaine when applied to specimens in KCl, N/500, induce a flowing of the protoplasm toward the pipette. This does not result in the formation of a pseudopod and the original direction of streaming is usually maintained (fig. 7; C).

#### *5. Non-electrolytes*

If to amebas in KCl, N/500, there is applied M/5 sucrose (cane sugar) to any point on the anterior two-thirds of the surface, no appreciable response occurs until about ten seconds have passed then streaming is suddenly reversed and pseudopods are formed successively in almost every direction (fig. 8; 1a, 1b, 1c, 1d, 1e, 1f). Antero-posterior differentiation is obscured for forty or more minutes, then the specimen assumes gradually a more normal appearance until within  $1\frac{1}{2}$  hours it appears as it did before the sugar was applied. The response to M/500 sugar is less pronounced and is usually indicated by a single reversal in streaming and the formation of a pseudopod near the posterior end when the sugar is applied at the anterior end, and by the formation of a pseudopod on the opposite side with or without reversal of streaming when the sugar is applied at the sides.

If methyl, ethyl or propyl alcohol in concentrations of 95 per cent and 50 per cent, is applied at any point on the surface of amebas in KCl, N/500, a hyaline blister is formed at the point of application, into which frequently a few endoplasmic granules flow (fig. 9; 1, 2, 3). The direction of streaming then is usually changed to one opposite that taken originally. The blister is resorbed. In lower concentrations when the application is at the anterior end, streaming is reversed, but no blister is formed and when the application is at the sides, streaming is diverted from its original direction by the formation of a pseudopod on the side opposite the point of application.

Methyl alcohol (95 and 50 per cent) induces the formation of blisters more readily than either ethyl or propyl alcohol when

used in the same concentrations and applied to specimens in KCl, N/500. None of the alcohols mentioned produces these results when applied to specimens in culture fluid, blisters are not formed and streaming is not reversed but one or more pseudopods are put forth which persist so that streaming is continued in a direction toward the source of stimulation (fig. 9; , A, 4, 5, 6).

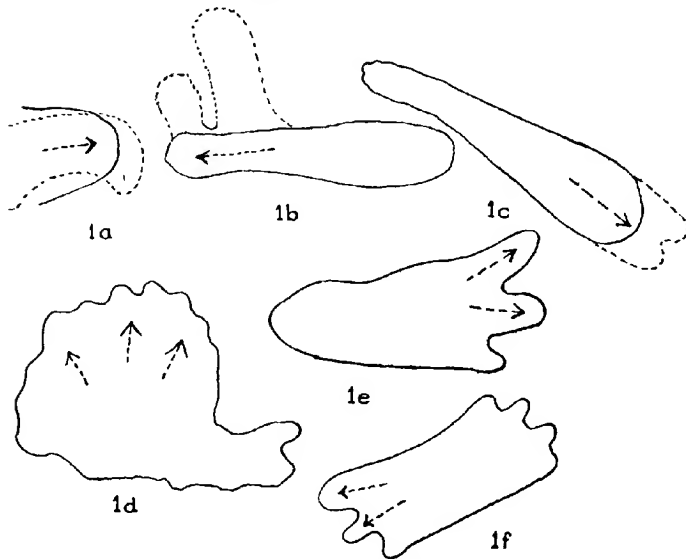


Fig. 8 Sketches of a single specimen illustrating reactions of Ameba to M, 5 cane-sugar with Ameba in KCl, N 500. Symbols as in figure 2.

Methyl alcohol 20 per cent, ethyl alcohol 16 per cent, and propyl alcohol 5 per cent when applied to specimens in KCl, N 500, cause streaming to be diverted away from the source of stimulation through the formation of a pseudopod (fig. 9; B, 1, 2) but no blister is formed.

#### 6. *Potassium cyanide*

If potassium cyanide, N 100, N 500, N 1000, is applied to specimens in culture fluid either at the anterior end or laterally the protoplasm flows toward the pipette for a brief moment

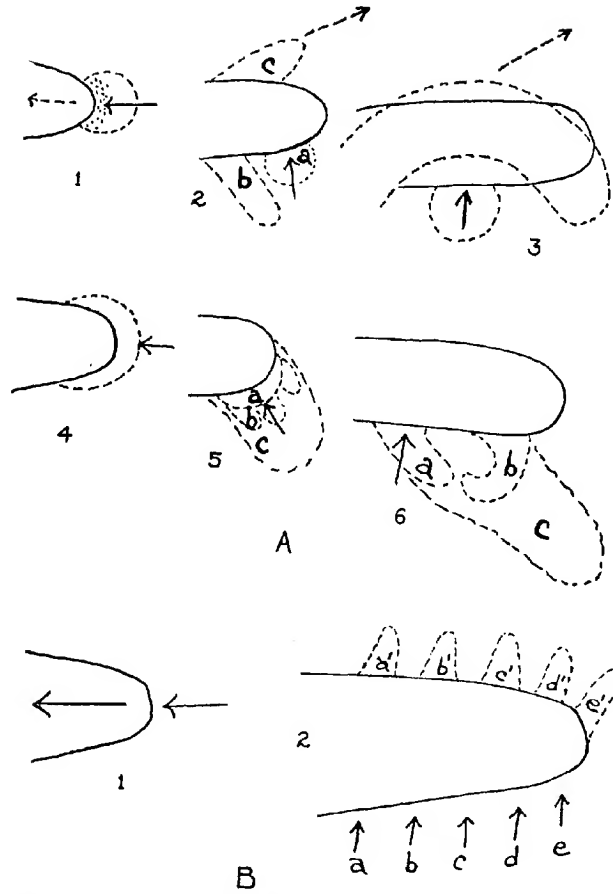


Fig. 9 Sketches illustrating reactions of Ameba to local application of alcohol. Symbols as in figure 2.

A, Methyl and ethyl alcohol, 95 per cent and 50 per cent, 1 to 4 with Ameba in KCl, N/500; 4, 5, 6, with Ameba in culture fluid.

B, Methyl, alcohol 20 per cent, ethyl alcohol 16 per cent, and propyl alcohol 5 per cent, with Ameba in KCl, N/500.

after which it flows in some other direction away from the pipette (fig. 10; A, 1, 2). The response is similar to that given when either sodium or potassium hydroxide is used as the reagent. When, however, KCN, N/100, is made neutral by the addition of HCN with phenolphthalein as indicator, the response consists in an immediate and vigorous flowing away of the protoplasm from the pipette (fig. 10; B, 1a, 1b; 2a, 2b).

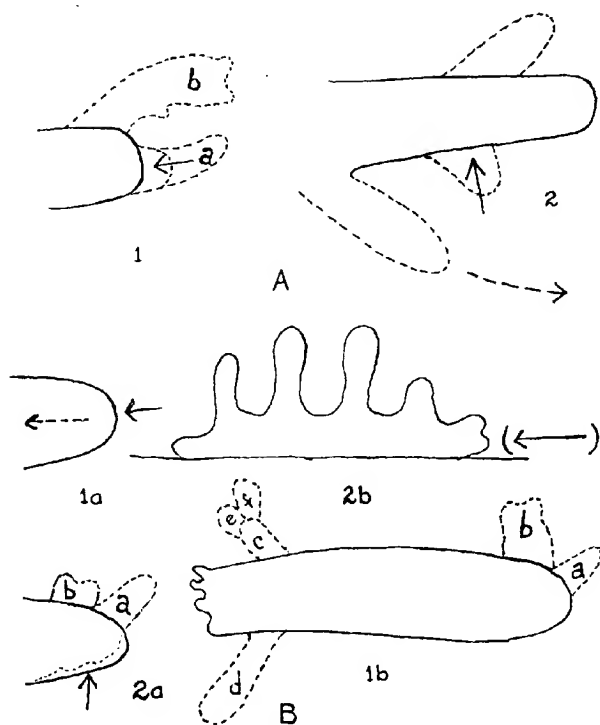


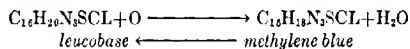
Fig. 10 Sketches illustrating reactions of Ameba to local application of KCN. Symbols as in figure 2.

A, N/100 KCN with Ameba in KCl, N 500.

B, N/100 KCN made neutral with HCN,—Ameba in KCl, N 500.

7. *The leucobase of methylene blue*<sup>2</sup>

The leucobase of methylene blue was used in order to compare the effect of the removal of oxygen locally from the surface of Ameba with that obtained by using potassium cyanide, which is believed by some to inhibit oxidation; and with that obtained by using hydrogen peroxide which is a well known oxidizing agent. After the leucobase is oxidized to the dye, it has no visible effect on Ameba. The change from the leucobase to the dye is brought about by the addition of oxygen to the hydrogen in the molecule of the leucobase thus:



The leucobase in solution is light green in color and each stage in oxidation is indicated by an increase in blueness until at complete oxidation it is the color of the well known non-toxic and physiologically neutral dye methylene blue. When it is applied to Ameba, presumably the oxygen it absorbs, reduces the oxygen pressure at the point of application and therefore it is to be expected that any effect resulting from its application must be owing to a reduction of oxygen pressure.

The leucobase was used in various stages of oxidation. In the first and least oxidized stage the most pronounced effect is obtained. When this is applied at the anterior end, there is a cessation of ectoplasmic advance followed immediately by a reversal of streaming. The ectoplasm becomes rapidly bluish and contracts and the endoplasm becomes heaped at the posterior end. Then streaming is again reversed and the endoplasm piles up at the anterior end for a few seconds; then returns to the posterior end, where a pseudopod is formed to one side of the posterior folds extending obliquely backward (fig. 11: A, 1a,

<sup>2</sup> The leucobase of methylene blue and of malachite green was prepared especially for the writer by L. M. Hussey, Dept. Biochem. Res., The Harmer Laboratories Co., Lansdowne, Pa. Only the leucobase of methylene blue could be used in the present investigation. The writer is very grateful to the Harmer Laboratories Co. in general, and to Mr. Hussey in particular, for the very great courtesy and interest shown in the preparation of the above-mentioned leucobases.

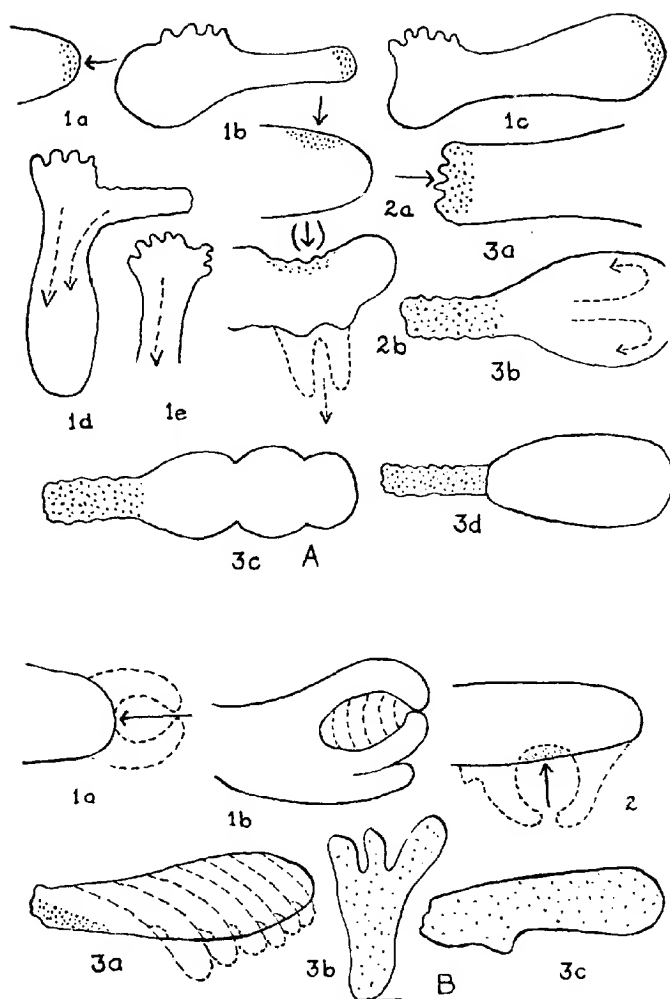


Fig. 11 Sketches illustrating reactions of Ameba to local application of the leucobase of methylene blue. Symbols as in figure 2. Stippled areas indicate how far the reagent spread by diffusion and the amount of surface primarily concerned in the reaction.

A, a solution of the leucobase applied when but very slightly oxidized.

B, a solution of the leucobase applied when about three-fourths oxidized.

1b, 1c, 1d, 1e). The original anterior end, now still quite blue, becomes slowly incorporated in the folds of the original posterior end as streaming continues in the new direction. Lateral application causes a contraction of the ectoplasm at the point applied and almost simultaneously one or more pseudopods are formed on the side opposite (fig. 11; A, 2a, 2b). Application at the posterior end seems to cause the endoplasm to flow forward faster than the ectoplasm can advance owing to a too rapid contraction of the ectoplasm at the posterior end, so that the specimen appears club-shaped. When the reagent diffuses over about one-sixth of the posterior end, the ectoplasm contracts, forcing the endoplasm forward until the anterior half is greatly distended and the posterior half has drawn together until it becomes filamentous (fig. 11; A, 3a, 3b, 3c, 3d). In some experiments the posterior half was covered by the leucobase. In these the response differs from the one just described in that the anterior end becomes more distinctly club-shaped and the specimen is detached from the substratum while fountain currents are seen in the endoplasm. These currents continue for a minute or more, then the ameba is constricted into two or more segments after which the fountain currents continue. Within ten minutes the ameba becomes reattached and locomotion continues normally. The appearance just described is seen elsewhere only in specimens immersed in an acid. It nearly always precedes death caused by acids.

If the leucobase is about three-fourths oxidized when it is applied to any point on the surface of the anterior half of the ameba, it causes the formation of a concavity at the point of application. Then two or more pseudopods are extended on either side of the pipette. These curve toward the pipette, meet each other and fuse. A sheet of ectoplasm spreads between these two from above so that a protoplasmic cup is formed (fig. 11; B, 1a, 1b, 2). When the posterior end is stimulated there is a slight contraction at this end and a corresponding enlargement of the anterior end. Sometimes a lateral pseudopod arises seemingly in compensation for the inability of the processes at the anterior end to meet the pressure from behind, i.e., the ectoplasm does

not seem capable of extension beyond a certain point at any one local area, so that if the processes at the posterior end are accelerated without corresponding acceleration at the anterior end, more than one pseudopod develops. Stimulation laterally near the posterior end causes the anterior end to curve toward the pipette thus forming a crescent. Since streaming does not seem to be affected, the ameba begins to move in a sort of spiral with the anterior tip curving more and more toward the posterior. After each successive change in curvature a small hyaloplasmic pit is formed slightly anterior to the middle region and on the inside of the crescent. When the region of bending changes, the endoplasmic granules fall into this clear, pit-like area (fig. 10; B, 3a). This process is repeated with each successive change in advance. It remains this way for 3 or more minutes during which there is little streaming. Within 8 to 10 minutes it falls over and reattaches (fig. 11; B, 3c). The stimulated area, at first blue, becomes no longer distinguishable as such, owing to the diffusion of the color over the entire specimen. The color changes slowly to lavender and finally disappears altogether at the time the specimen resumes normal locomotion.

### *S. Hydrogen peroxide*

Hydrogen peroxide, as is well known, is an oxidizing agent and one which therefore would be expected to affect Ameba quite differently from a reducing agent like the leucobase of methylene blue. Accordingly M 100,  $\text{H}_2\text{O}_2$ , was applied to specimens in culture fluid and in KCl, N 500. When it is applied at the anterior end streaming stops within 5 seconds. Within 10 seconds there is a reversal of streaming and a pseudopod then forms either at the posterior end, or along the upper surface, so that it extends up into the solution (fig. 12; 1 a, b, c, d). In some instances specimens remain elongated and motionless for several minutes without the formation of any pseudopods; in others, two parallel endoplasmic currents meet each other at the anterior end and pass inward and backward, i.e., just the opposite of the fountain currents described above. Lateral application induces the formation of a pseudopod on

the opposite side; or a reversal in streaming, after which the ameba becomes detached and greatly elongated (fig. 12; 2 a, b). There is no contraction or expansion at the point where the reagent is applied. Application at the posterior end is without effect unless the reagent is used undiluted, in which case a clear hyaline area develops between the ecto- and endoplasm at the point applied. This clear area then extends uniformly around the specimen so that it appears cylindrical with a conspicuous hyaline area between ecto- and endoplasm. Within 15 to 20 minutes this clear margin disappears, but the ameba remains unattached and practically inactive for several hours. All amebas to which  $H_2O_2$  was applied, tend within a few minutes after the application, to become inactive and sausage-shaped.

#### GENERAL PRESENTATION OF RESULTS

Of the responses induced by chemicals differing visibly from those manifest in the normal extension and withdrawal of pseudopods, the following should be noted: In the response to acids (particularly at the anterior end) the protuberance or pseudopod initiated is extended eruptively as though some pressure at the posterior end were causing the ectoplasm to distend in excess of that normally observable. If the acid is strong, N/10 to N/100, the response is frequently limited to the formation of a protuberance, after which further expansion is seemingly limited by a hardening or gelation of the surface. The eruptive character becomes less pronounced with low concentration of the acid, but it never entirely disappears. In the responses of *Ameba* to neutral potassium cyanide and the leucobase of methylene blue the ectoplasmic area stimulated visibly contracts before a pseudopod is initiated on the opposite side, or away from the source of stimulation. The place or origin of this pseudopod seems determined somehow by the contraction.

The concentration of any chemical used in this investigation rarely seems to affect the essential character of the response. Alcohol in high concentration is perhaps an exception, in which case a blister forms at the stimulated area which resembles

neither a protuberance nor a pseudopod such as is put forth, for instance, in the application of acids. If the response consists in the formation of a protuberance away from the diffusing reagent in a given concentration, this is found to be true of all concentrations in which any response is given. The difference in response to different concentrations of the same chemical is one of degree, not of kind, i.e., a protuberance is not extended toward the pipette in one concentration and away from it in another. The response varies in magnitude depending on the concentration, but is not essentially different. Different responses are obtained to the same chemical by using different media.

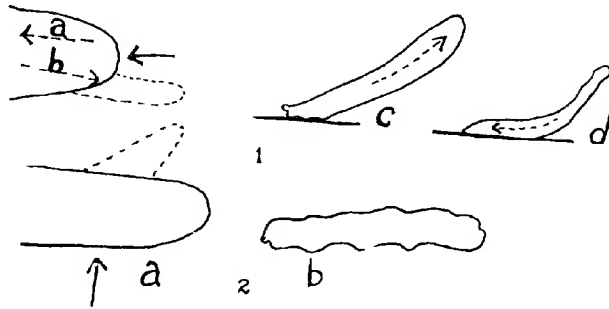


Fig. 12. Sketches illustrating reactions of Ameba to local application of hydrogen peroxide N/100. Symbols as in figure 2.

The visible effects of chemicals on Ameba are obviously those which are observable either in the extension of a pseudopod toward or away from the diffusing chemical. Some of these are temporary, others are of such permanence as to determine the subsequent direction of locomotion. The extension of a pseudopod away from the source of the diffusing chemical is preceded in many instances by a visible contraction of the ectoplasm at the point of stimulation before the pseudopod is initiated on the opposite side. Of these effects, only those manifest in the responses which lead the entire organism to travel toward or away from the source of stimulation are to be regarded as positive or negative respectively.

The following chemicals induce Ameba in the majority of the experiments to travel toward the source of their diffusion (positive responses):

<i>Chemical</i>	<i>Concentration</i>	<i>Medium</i>
NaOH or KOH	N, 2000	Culture fluid
NH <sub>4</sub> Cl, NH <sub>4</sub> SO <sub>4</sub>	N, 1 and N, 100	Culture fluid and KCl
Quinine and cocaine	M, 500, M, 50, respectively	KCl, N, 500.

The following chemicals induce Ameba in the majority of the experiments to travel away from the source of their diffusion (negative responses):

<i>Chemical</i>	<i>Concentration</i>	<i>Medium</i>
Acids	N, 1, N, 500	KCl, N, 500
HCl; NH <sub>4</sub> Cl, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and CO <sub>2</sub>	N, 1 to N, 1000; N, 1 and N, 100; saturated, respectively	NaCl, N, 500
NaCl; neutral KCN; methyl and ethyl alcohol; leucobase of methylene blue; and H <sub>2</sub> O <sub>2</sub>	N, 1 and N, 100; N, 100; 95, 50, 20 per cent variously oxidised; and N, 100, respectively	KCl, N, 500
Quinine and cocaine	M, 500 and M, 50 respectively	Culture fluid
Quinine hydrochloride	M, 500	Culture fluid
KNO <sub>3</sub> and KOH	N, 300 and N, 4000 respectively	HCl, N, 10,000

The following chemicals cause the extension of a blunt or a finger-like protuberance toward the region stimulated:

<i>Chemical</i>	<i>Concentration</i>	<i>Medium</i>
HCl, HNO <sub>3</sub> , H <sub>2</sub> SO <sub>4</sub> , HCN, CO <sub>2</sub> , and oxalic acid	N, 1 to N, 20,000	KCl, N, 500, culture fluid
NaOH, KOH	N, 100 to N, 4000	KCl, N, 500, culture fluid
NH <sub>4</sub> Cl, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	N, 1 and N, 100	KCl, N, 500, culture fluid
NaCl, KCl, NH <sub>4</sub> Cl, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , BaCl <sub>2</sub> , SrCl <sub>2</sub> , MgCl <sub>2</sub>	N, 100	Culture fluid
KCN (basic)	N, 100 and N, 500	KCl, N, 500, culture fluid
Quinine and cocaine (alkaloid)	M, 500, M, 50 respectively	KCl, N, 500
Methyl and ethyl alcohol	50 per cent	Culture fluid
HCl	N, 10,000	KOH, N, 4000

The following chemicals cause the extension of a blunt or a finger-like protuberance away from the region stimulated:

<i>Chemical</i>	<i>Concentration</i>	<i>Medium</i>
KCl, NaCl, $\text{NH}_4\text{CO}_3$ , KCN (neutral)	N/100	KCl, N/500
Morphine and strychnine	M/50	KCl, N/500
Papaverine hydrochloride	N/100	KCl, N/500
Sucrose (cane-sugar)	M/5 and M/500	KCl, N/500
Methyl, ethyl and propyl alcohol	20, 16, 5, per cent	KCl, N/500
Leucobase of methylene blue	in various stages of oxidation	KCl, N/500
$\text{H}_2\text{O}_2$	N/100	KCl, N/500
Quinine and cocaine (alkaloid)	M/500, M/50 respectively	Culture fluid
Quinine hydrochloride	M/500	Culture fluid
KOH, $\text{KNO}_3$	N/4000, N/300 respectively	HCl, N/10,000
$\text{NH}_4\text{Cl}$ , HCl	N/100, N/10,000 respectively	NaCl, N/500

In the following columns is indicated the probable local effect and the general effect:

1. *Local effect on the ectoplasm*

Acids: liquefying-gelating

Hydroxides: Liquefying

Salts: Liquefy or gelate with corresponding expansion or contraction of the area to which salt is applied

Alkaloids:

Non-electrolytes: a) sugar,—osmotic effect; b) alcohol,—liquefying

Potassium cyanide: a) basic,—liquefying. b) neutral,—gelating

Leucobase of methylene blue: Gelating

Hydrogen peroxide: Liquefying

2. *General effect on the organism*

Protuberance initiated toward reagent after which Ameba moves away from it.

Protuberance initiated without appreciable change in the direction of locomotion unless stimulus applied at the anterior end.

Travels toward or away from salt depending on the salt, the concentration and the medium.

Variable.

a) If strong,—streaming alternately in all directions; if weak, Ameba travels away from stimulating agent. b) Ameba travels toward reagent in culture fluid and away from it when in KCl, N/500.

a) Slight to no effect on locomotion. b) Ameba travels away from reagent.

Ameba travels away from reagent when it is unoxidised but forms encircling protuberances around stimulated area when about three-fourths oxidised.

Ameba travels away from reagent and becomes inactive.

## DISCUSSION

Jennings ('04), who ascertained the local responses of *Ameba* to various substances, found as a result of applying certain chemicals locally that *Ameba* travels in general in a direction opposite from that in which the chemical is applied. He says (p. 187): "I believe there is no instance of positive chemotaxis in *Rhizopoda* where the nature of the active substance is known and the reaction was controlled experimentally." He describes a number of positive reactions observed in connection with feeding, i.e., where the *ameba* travels toward the food. These, he thinks, should be attributed partly to chemical stimuli. In substantiation of this statement is the fact that the writer obtains with dilute acids—particularly carbonic—reactions markedly similar to those manifested in the ingestion of motile protozoa as described by Kepner and Taliaferro ('13) and Kepner and Edwards ('17). The writer is inclined to the belief that this type of reaction is conditioned in part at least by a local increase in the H-ion concentration. Schaeffer ('17, p. 226) concludes from his experiments with egg albumin and globulin, and egg albumin and silicic acid, that they "show clearly that the presence of a substance in solution only is not sufficient to attract *ameba*, nor to cause ingestion, but that the substance must be actively diffusing from a definitely localized region."

In connection with the local response to acids, it should be borne in mind that in all the experiments, the initial phase is characterized by the formation of a protuberance either slight or extensive followed by a cessation of ectoplasmic expansion, and if the acid is sufficiently dilute, by the extension of two or more pseudopods on either side of the region to which the acid was applied. Aside from the similarity of this type of response to those observed in certain feeding reactions already referred to, the probable state of the ectoplasm should be considered. Hyman ('17) is of the opinion that some local change in the ectoplasm of a liquefying character which is dependent upon metabolism is responsible for the extension of a pseudopod; and Leo Loeb ('20) while agreeing with Hyman, holds further that a

change in hydrogen ion concentration is also concerned. Jacobs ('22) shows that carbon dioxide is an efficient agent for changing the consistency of the protoplasm reversibly in either direction, the exact effects produced depending on the concentration of the gas, the material experimented upon, the time of exposure and the presence or absence of other dissolved substances in the medium. He ejected distilled water saturated with carbon dioxide through a very fine capillary against the surface of Ameba and observed that pseudopods were put forth in the direction of the capillary. The changes in the protoplasm manifest in the effects induced by the carbon dioxide he suggests are dependent upon the absolute concentration of the  $\text{CO}_2$  rather than on the pH of the solution. He consequently confirms the results obtained by the writer ('21) who, in the local application of acids to amebas, observed that the response consists in the formation of a protuberance, the equivalent perhaps of a pseudopod, which arose probably by virtue of an initial liquefaction at the point to which the acid was applied. Thus the results of Jacobs in so far as they apply agree substantially with those of the writer.

Concerning the nature of the changes at the surface of Ameba by means of which pseudopods are extended or withdrawn and locomotion accomplished, the observations of early investigators led them to postulate first, movement owing to the presence of some physically unexplained contractile substance and second, movement owing to local changes in surface tension. The latter has received the wider attention. Surface tension phenomena as postulated by Bütschli and Rhumbler and others in explanation of ameboid movement are such as can occur only at liquid interphases. Clearly, the results obtained in this investigation indicate that the surface phenomena manifested are not comparable to those obtainable in the surface films of ordinary liquids. To what extent the forces of surface tension factor in ameboid movement when these forces are understood in connection with such a colloidal state of matter as protoplasm represents, it is of course not possible at present to say. That the surface of Ameba is for the most part a gel-like solid seems clear

from the fact that it resists going into solution in reagents which either before or after its rupture have a liquefying effect upon it. This is plainly seen in the action of sodium or potassium hydroxide, which has a liquefying effect on the surface in both instances. In fact, specimens which rupture when immersed in a strong solution of KOH so that the endoplasm streams out and disappears, are seen with their ectoplasmic membranes preserved but somewhat shrunken and collapsed for twenty or more minutes after the rupture has occurred and the endoplasm has become dissolved. The surface membrane is ruptured whenever NaOH or KOH, N, 1, is applied locally to amebas. The endoplasm streams out through the small aperture until only the ectoplasmic membrane remains unless the hydroxide *is neutralized or the Ameba moved away from the vicinity where the hydroxide was applied.*

It is obvious, consequently, that the surface of Ameba differs markedly from its interior and from its environment. It is this difference that determines the character of the local changes which chemicals induce when applied locally to the surface. Concerning this difference of the surface layer of cells in general, Starling ('15) says: "Since it differs from the rest of the protoplasm in the changes to which it is subject, it must also differ in its chemical composition apart altogether from the factors which . . . determine molecular differences between the surface and the interior of any colloidal solution."

Mathews (21) suggests that "It is probable that the surface membranes of all cells are produced in part at least by the process of secretion from a solution upon its surface, of substances which lower the surface tension of the interphase and are sufficiently insoluble. The quick coating over of ameba's pseudopodia looks as if such a process occurred." He suggests further that the reduction of surface tension by the passage of substances that lower surface tension to the surface, is a result of their accumulating there—not the cause. The cause he says is probably that the cohesion of the solvent molecules is greater than the adhesion of the solvent for solute or the cohesion of the solute, and as a consequence the solvent molecules are drawn in from the surface, leaving the solute concentrated in the surface.

It is probable that many factors influence the accumulation of substance at the surface, both internal and external. The process of secretion may not occur uniformly owing to limiting factors of metabolism, or in conjunction with changes in the medium and hence condition the formation of pseudopods. It is, of course, not possible to say what are the actual factors at work when pseudopods are put forth or withdrawn and what conditions the stickiness of the surface by virtue of which attachment is effected. Some of the probable factors may be more definitely localized in the results of subsequent investigation.

#### SUMMARY

1. *Acids*. When acids diffuse against the anterior three-fourths of an ameba in KCl, N, 500, the response consists in the formation at the point where the acid comes into contact with the surface of a short blunt protuberance if the acid is strong and a finger-like protuberance if it is weak. There is no appreciable response when acids diffuse against the extreme posterior end. The response occurs immediately when acids are applied in high concentrations and within 5 to 10 seconds when they are applied in low concentrations. As a rule neither the short blunt nor the longer more finger-like protuberance persists. Streaming is usually reversed but when the acid is sufficiently dilute and is allowed to diffuse laterally, the protuberance initiated does not affect appreciably the original direction of locomotion.

The type of response just described occurs also when acids are applied to amebas in culture fluid or in dilute KOH, but when they are applied to amebas which have been in NaCl, N/500, for 18 or more hours, the response consists in a slight contraction of the region to which the acid was applied and an almost simultaneous change in the direction of locomotion through the formation of a pseudopod on the side opposite the source of stimulation.

The formation of protuberances in response to acids seems to be conditioned by a liquefaction of the ectoplasm. This is

followed by a gelation or hardening of the ectoplasm after which it visibly contracts and soon the protuberance formed disappears. The initial liquefaction and the subsequent gelation are more rapid in strong than in weak concentrations.

2. *Hydroxides*. When the hydroxide of sodium or potassium diffuses against the anterior three-fourths of an ameba in KCl, N/500, or in culture fluid, the response consists in the gradual formation at the point of application of a broad protuberance when the hydroxide is strong and of a more slender finger-like protuberance when it is weak. There is no appreciable response when it is applied at the extreme posterior end. The response occurs usually within 1 to 5 seconds. The protuberances formed do not as a rule persist so as to function as pseudopods except occasionally when the amebas are in culture fluid. The original direction of locomotion is generally not affected appreciably by the application of the hydroxide. The length of time the stimulus is applied has but little effect on the magnitude of the response. Certain media cause the response to differ as for example, when either NaOH or KOH is applied to amebas in dilute HCl or NaCl (in the latter after 18 or more hours), the response consists in a slight contraction of the region to which the hydroxide was applied and a simultaneous change in the direction of locomotion through the formation of a pseudopod on the side opposite the source of stimulation.

The response to NaOH and KOH is apparently conditioned by a liquefaction of the ectoplasm but without subsequent gelation as in the case of acids.

Ammonium hydroxide induces a response which consists of an eruptive advance of the ectoplasm toward the source of stimulation followed immediately by a cessation of streaming and the assumption of a somewhat spherical form. The response seems to be owing to an equal action of both the  $\text{NH}_4$ -ion and the OH-ion.

3. *Salts*. When salts in concentrations of N/1 or N/100 diffuse against the surface of an ameba in KCl, N/500, the response consists usually in a contraction of the ectoplasm at the point stimulated followed by a bending of the ameba away

from the source of stimulation and (in the application of most of the salts used) the formation of one or more pseudopods on the side opposite the stimulated region. If the amebas are in culture fluid, the response consists in the formation of a protuberance at the point stimulated which in many instances persists as a functional pseudopod, in others the original direction of locomotion is not at all or but little affected. Neutral ammonium salts have the most marked effect on the ectoplasm of amebas in either KCl or culture fluid causing in most instances the formation of a pseudopod at the point at which they are applied. The pseudopod thus initiated usually persists and determines the subsequent direction of locomotion. If, however, they are applied to an ameba in NaCl, the ectoplasm contracts at the point stimulated and a pseudopod forms on the side opposite the stimulated region and the ameba travels away from the source of stimulation.

The response to salts is in general variable and depends upon the composition of the salt; the concentration; and the medium in which the amebas are when stimulated. The concentration affects chiefly the magnitude of the response. Changes in permeability seem to condition this type of response.

4. *Alkaloids*. When alkaloids are applied to amebas in KCl, N/500, or in culture fluid, the response consists in a slight contraction of the ectoplasm in the region where the reagent was applied, and the formation of a pseudopod on the opposite side if the reagent is applied laterally or away from the reagent if it is applied at the anterior end. Quinine and cocaine induce the formation of protuberances toward the pipette. Some of these persist thus determining the direction of locomotion. The latter is true when the specimens are in KCl, N/500, but it is only occasionally true when they are in culture fluid.

5. *Non-electrolytes*. a) Cane-sugar, M, 5 causes so many and so rapid changes in streaming that locomotion is practically stopped. The amebas become normal within 30 or more minutes. the effect is probably an osmotic one. b) Alcohol 95 and 50 per cent produces a hyaline blister at the point to which it is applied, when the specimens are in KCl, N, 500, and then streaming is

diverted away from the pipette; but when applied to specimens in culture fluid no blisters are formed and streaming is usually diverted toward the pipette. Weak solutions of alcohol applied to specimens in KCl, N/500, cause streaming to be diverted away from the pipette, but no blisters are formed. On specimens in KCl alcohol seems to have a gelating effect and on specimens in culture fluid a liquefying effect.

6. *Potassium cyanide* applied without being neutralized with hydrocyanic acid, produces a small protuberance which does not persist. When it is applied after it has been neutralized with HCN, the ectoplasm contracts at the point where it was applied and streaming is simultaneously diverted away from the pipette. The first type of response is apparently conditioned by the OH-ion, the second by the CN-ion.

7. *The leucobase of methylene blue*, if applied before it has undergone appreciable oxidation, causes violent contraction over the area to which it is applied which at the same time becomes blue. Streaming stops momentarily and is then resumed in a direction away from the source of the diffusing leucobase. When it is applied after it has undergone considerable oxidation, there is a slight contraction of the surface to which it is applied and two or more pseudopods arise on either side of the area stimulated and curve toward each other gradually forming a protoplasmic cup. There is no indication that initial liquefaction precedes gelation, but rather that the action is one of gelation only.

8. *Hydrogen peroxide* induces cessation of streaming, followed almost immediately by locomotion away from the reagent and, within a minute, by detachment from the substratum and inactivity. The effect on the ectoplasm is not obvious.

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# STUDIES OF THE PELAGE PHASES AND OF THE NATURE OF COLOR VARIATIONS IN MICE OF THE GENUS PEROMYSCUS

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FIFTY-SEVEN FIGURES

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## 1. INTRODUCTION

In mice of the genus *Peromyscus*, variations in color are frequently quite marked. This is true not only of material from different localities but also of individuals which occur in the same region. In several species of the genus, these color differences have led to the description of pelages as in 'buff' as contrasted to 'dark' or 'gray' phases. According to Osgood, "a sort of dichromatism is found in *P. m. blandus*, one phase being vinaceous gray and the other ochraceous buff. A few other forms, as *sonoriensis* and *coolidgei*, are slightly and less commonly dichromatic" (p. 16).

According to the same writer, variations of an extremely local and sporadic nature are found especially among western representatives of the genus. These variations are attributed to the differences in environmental conditions encountered by the species in diverse parts of their ranges.

The common occurrence and the amount of local and geographic variation in various characters, but especially in color has naturally led students of the group to predicate a large degree of plasticity in response to different conditions.

In addition to the differences in color supposed to be due to environmental differences are those phases which characterize the live-cycle and the seasonal variations resulting from fading and abrasion.

During the past eight years, a series of genetic and distributional studies involving several geographic races of *Peromyscus* have been in progress at the Scripps Institution for Biological Research, under the direction of Prof. F. B. Sumner. In the course of these experiments it became apparent that a more exact knowledge of the nature of color variations within the groups used in the experiments was necessary for the study of racial differences as regards color. It is the purpose of the present paper to present the results thus far obtained by the writer in a study of these intra-racial differences in color and to describe some kindred studies growing out of the main problem.

## 2. ACKNOWLEDGMENTS

It is with pleasure that I acknowledge the many helpful suggestions and criticisms of Prof. F. B. Sumner, of the Scripps Institution. I am also very much indebted to Prof. Joseph Grinnell, director of the Museum of Vertebrate Zoology at Berkeley, for reading this paper in manuscript, and for placing the facilities of the museum at my disposal. Finally, I wish to express my deep appreciation of the privilege I have enjoyed, during my three years at the Scripps Institution, of frequent conferences with the director, Prof. W. E. Ritter, who has also read this paper and offered helpful criticisms.

## 3. MATERIAL

The material used for the most part was the local La Jolla form of *Peromyscus maniculatus gambeli*. During the course of the work, over five hundred mice of this subspecies were trapped on, or within two miles of the Institution grounds. In all, about four hundred skins were prepared and preserved for study. More than five hundred individuals of this same subspecies were reared by me in the murarium to furnish living material for certain phases of the investigations. Smaller stocks were kept of *P. eremicus fraterculus*, *P. californicus insignis* and *Mus. musculus*. In addition to this material I had access to Professor Sumner's cultures of *P. m. gambeli*, and of the transplanted races, *P. m. sonoriensis* from the Mohave Desert, and *P. m. rubidus* from the humid coast belt of northern California. In all, a living population varying in number from one thousand to fifteen hundred was generally available for observation.

In addition to the above, a total of over three thousand skins in the collections of the Museum of Vertebrate Zoology were examined as follows: *P. m. hylaeus*, 223 (adult, adolescent and juvenal); *P. m. macrorhinus*, 38; *P. boylei boylei*, 110; *P. m. sonoriensis*, 735; *P. truei gilberti*, 260; *P. boylei rowleyi*, 510; *P. truei truei*, 70; *P. californicus californicus*, 88; *P. californicus insignis*, 170; *P. eremicus eremicus*, 255; *P. eremicus fraterculus*, 95; *P. m. austerus*, 233; *P. m. rubidus*, 123; *P. m. gambeli*, 485.

## 4. METHODS

*1. Preparation of skins*

In the preparation of skins for study I have followed Sumner's method.<sup>1</sup> The body is removed through a slit in the skin extending from the base of the tail along the median line to the throat. After removal of all fatty tissue, the inner surface is treated with powdered arsenic. The skin is next placed on a stretcher and subjected to equal tension by means of 20-gram weights attached to flexible wires which run on pulleys, and which in turn are attached to the skin by means of small spring clips at eight different points, viz., the four limbs, the tip of the snout, base of the tail and at the middle points of the sides. After an interval of about fifteen minutes, the skin while still stretched taut is pinned out on a small board where it is left for a week. The skin along with others is next placed in a bath of gasoline or benzine for a day or two. It is then transferred to a second bath for the same length of time. As a final step in the process, it is removed from the second bath and exposed to the air until entirely free from the fluid, when the hair is brushed smooth. Subsequent exposure to light is avoided except for purposes of examination.

Where large intergrading series are to be studied, this method of preparation is more satisfactory than the method used in the preparation of conventional museum skins which are stuffed with cotton. Both the dorsal and ventral surfaces may be seen in one plane. Differences due to unequal stretching, wrinkling, and high lights and shadows are eliminated. As the skin goes through the gasoline baths, oil and dust particles are removed from the hair. This is an important step in the process since not infrequently the appearance of the skin is noticeably different after being immersed in gasoline.

*2. Color analysis of skins*

The terminology used in the description of colors is that of Ridgway's Color Standards and Color Nomenclature ('12).

<sup>1</sup> Journal of Experimental Zoölogy, vol. 7, no. 1, August, 1909, p. 101.

While this method of color description meets most of the needs of the naturalist, for an intensive study of color variations within a race and of racial differentiation as regards color, it is highly desirable to be able to deal quantitatively with the differences in question.

In collaboration with Professor Sumner, a satisfactory method for the quantitative expression of the color composition of a pelage has been devised. The method consists in a simple adaptation of the color wheel as used in physical and psychological laboratories.

The apparatus consists of two aluminum discs, each of which is attached directly to the shaft of a small electric motor. Color sectors are mounted on one disc while the skin which is to be analyzed is held in place on the other by means of a diaphragm which is attached to the disc by bolts. The area of the skin to be analyzed is exposed to view by a circular aperture in the centre of the diaphragm. When a skin in this position is rotated at a high rate of speed, the effect of centrifugal force is such as to cause displacement of the individual hairs in all directions from the center. This difficulty is obviated by covering the exposed surface of the skin with a very thin transparent gelatin film.

The rotation of the discs is controlled by foot rheostats. These discs are rotated at a speed sufficient to produce perfect blending of the colors.

In the analysis of *Peromyscus* skins it was found necessary to use black, white, orange and yellow. It was found practicable to eliminate one of the variables by using orange and yellow in the constant ratio of 1:2.

As determined by duplicate analyses, the average difference (expressed as a per cent of the lesser of the two readings) in the determinations of black, which is by far the largest color component, is 0.6 per cent. The average difference in determining the percentage of white plus orange-yellow is 2.7 per cent. Because of the relatively small percentages of white and orange-yellow and furthermore because, within narrow limits, the diluting effects of white and of yellow upon black are indistinguishable from one another, the differences in successive deter-

minations of white and of orange-yellow are somewhat larger. The average error for white is 7 per cent, for orange-yellow, 7.7 per cent. In every case, the color composition of the skins was determined a second time after an interval of at least one day and the average of the two readings was taken. All analyses were made during the day under fairly constant light conditions. The Milton Bradley color discs were used. Inasmuch as these colors fade somewhat after considerable exposure to sunlight, it was found necessary to keep the discs covered while not in use and to test them frequently with a second set kept continuously in the dark.

It is obvious that these errors are negligible where the series compared show little or no overlapping and the differences with which we are concerned are relatively large. However, in dealing with intergrading series it would be desirable to determine the color composition with greater precision.<sup>2</sup>

The elongated shape of the skin is such as to make it impracticable to determine the color composition of the whole dorsal surface. However, it is sufficient for purposes of comparison to select homologous representative areas for analysis. In the case of the juvenal, postjuvenal and adult skins of the buff and dark extremes, a circular area on the dorsum was selected having a diameter of 32 mm. and extending approximately to the ventral white on both sides, and from the base of the tail anteriorly. In the analysis of old and new pelages on opposite sides of the body, homologous regions on the flanks extending from the dorsal median line to the ventral white were compared.

### 3. *Microscopic structure of hair*

The color and arrangement of the pigments were studied to best advantage in unstained hair mounted in glycerin or balsam. The structure and surface striations of the cuticle were brought out by staining slightly with dilute picric acid. Air in the medulla was partially expelled by treating the hair with a weak solution of potassium hydrate.

<sup>2</sup> This result has recently been accomplished by Professor Sumner with the Hess-Ives tint photometer.

#### 4. *Study of molts*

In many cases, molts are of such an insidious character that it is necessary, in determining points of origin and directions of growth, to follow the process as the new hair comes through the skin. A molt may be well under way before any change is evident at the surface of the earlier coat of hair. The mice whose molts were being studied were etherized and the incoming pelage located by parting the overlying hair by blowing. These areas were then outlined on diagrams of the dorsal, ventral, and lateral surfaces of the body, which were printed on cards of convenient size, especially prepared for this purpose.

#### 5. *Skin pigmentation*

In the buff and dark extremes, a study was made of the correlation between the color of the pelage and the extent and intensity of the pigmentation of the soles of the feet. In preparation of the material for this study, Summer's ('19) method was used. The left feet were removed from the body soon after death and placed in 70 per cent alcohol, being later transferred to a mixture of equal parts of alcohol and glycerin. After being left in this fluid about a week, they were put into pure glycerin which serves as a very satisfactory clearing agent and preservative.

In order to determine the average condition and the variability in the two contrasted series, an arbitrary scale was adopted with five grades of pigmentation, a device similar to that used by Castle in grading the variability of the hooded pattern in rats.

#### 5. GENERAL FEATURES OF MOLT IN BIRDS AND MAMMALS

Before entering upon a discussion of molts in *Peromyscus*, it seems worth while, by way of introduction, to review the outstanding features of molts in birds and mammals, with especial reference to the rodents.

Both birds and mammals in general, undergo marked modification in appearance as the result of molting. Certain of these molts are to be regarded as adaptations to changes of season, while others are characteristic of certain stages in the life-cycle.

Although the general subject of molts has not been adequately investigated in either the birds or the mammals, it may be said that the ornithologists have pushed their investigations somewhat farther than have the students of the mammals. This is probably in part due to the fact that, as a rule, the changes in appearance induced by molt are much more obvious in birds than in mammals.

In many species of birds, the changes in appearance incidental to the transition from the juvenal to the adult are even more conspicuous than the changes due to season. The juvenal plumage, frequently so unlike that of the adult, is often regarded as a recapitulation, probably much modified, of an ancestral stage. In the words of Darwin, "As the young of so many species have been but little modified in color and other ornaments, we are enabled to form some judgment with respect to the plumage of their early progenitors. . . ." (p. 499).

No biologist appears to have placed greater dependence upon comparative studies of juvenal plumages, in attempting to unravel phylogenetic relationships than did Whitman ('04) in his studies of speciation in pigeons. Thus, for example, in tracing the evolution of various wing-bar patterns, he found transition stages in the juvenal plumage, linking together what appeared to be discontinuous variations in the adult.

Although it is true, as Darwin, Whitman and others have pointed out, that juvenal plumages often afford clues to ancestral relationships, nevertheless there are anomalous cases in which closely allied subspecies differ more in the juvenal than in the adult. Thus according to J. A. Allen ('94 a):

While in some species the young in first plumage bear a close resemblance in color to the adults, as in some of the Flycatchers, Jays, Chickadees, Vireos, etc., in other cases they are so totally unlike the adults as to be sometimes identifiable with difficulty even by experts, and only by structural characters rather than by plumage, as in various Warblers and Sparrows, as is well shown by the subject of the present illustration [the oven-bird]. The first plumage is thus often exceedingly characteristic, closely allied subspecies sometimes differing more at this early stage than at any later period. Its real significance, however, has as yet been little studied (p. 93).

A special study of the molts of birds such, for example, as that of Dwight ('00) on the Passerine birds of New York discloses a high degree of regularity in the process. The development of the various feather tracts follows a fairly definite sequence. The old feathers in any particular row tend to remain until the new ones in the adjacent row are sufficiently developed to assume their function. The remiges of the wings are nearly always shed in pairs. They drop out synchronously from the two wings or within a few days of each other, so that the power of flight is but slightly impaired by their temporary loss. The process is less regular as seen on the body, and the greater degree of regularity of the wings is evidently an adaptation for the preservation of the power of flight during the molting season. There are, however, exceptions to this rule. In the Anatidae and some other groups as well, the remiges are molted almost simultaneously and the power of flight is wholly lost for a time.

Although the molts of mammals, save in a few instances, have been described only in a rather superficial and incidental fashion, it seems certain that they are typically less regular in character than the molts of birds. Jackson ('15), in his monograph of the American moles, gives a much more complete discussion of pelages and molts than is usually found in monographs in this series.

Although generic differences in the molts of birds are well known, Jackson is the only writer, so far as I am aware, who has described generic differences in mammals, as regards points of origin and direction of growth of the new pelage. The nature of these differences is indicated in the following excerpts from his account.

Molting in *Scalopus* occurs more or less regularly in definite sequence on the different parts of the body, and the same order is followed in both the vernal and autumnal molts. The fresh pelage first appears on the breast and abdomen (Pl. I, fig. 1) and gradually replaces the old until the entire underparts, except the chin and throat, have molted; at this stage there is a sharp lateral line of demarcation between the new and the old fur (Pl. I, fig. 2); the fresh pelage gradually extends up over the back, generally encroaching upon the posterior part first and working forward toward the nose. The chin and throat in most individuals

retain the old pelage for several days after all the rest of the molt is complete. There are, of course, exceptions to this general order of molting but most of these occur in animals which are molting either earlier or later than normally, and the writer is inclined to believe that these variations are either due to retarded or stimulated physiological processes, or else result from injuries to the animal (p. 14).

The sequence of molting in *Scapanus* is less definite than in *Scalopus*. The differences of color, texture, and length of hair between the old and new pelages of *Scapanus* are usually slight; often the line of demarcation separating the two pelages is scarcely distinguishable, and seldom sharp as in *Scalopus*. The sequence of molting on the various parts of the body appears in a few cases to be not unlike that of *Scalopus*, the under parts molting first, followed consecutively by the sides and back. More frequently, however, the new pelage appears first on the head and throat, then works down over the nape and back, encroaching last upon the abdomen; or, as is shown most beautifully in a specimen of *Scapanus latimanus latimanus* from Petrolia, Cal., the new pelage may appear simultaneously in separate patches upon head, back, and rump (p. 15).

The new fur of *Condylura* generally appears first on the posterior part of the flanks, but the body sequence is inconstant; the molt on the flanks usually spreads forward and ventrally, while at the same time on the back fresh pelage replaces the old, which sloughs off in irregular blotches. Probably in most cases the ventral parts are in fresh pelage before the major portion of the back has molted; a small posterior rump patch is almost invariably the last to molt. The contrast between new and old pelages during the spring molt is marked; the autumnal molt, however, is often difficult to detect.

In the genus *Neurotrichus*, new pelage ordinarily first replaces the old on top of the head; this is soon followed by the molting of the posterior part of the back almost simultaneously with the beginning of the molting of the ventral parts on the throat and breast; the molting areas on the back increase in size and finally enclose each other; the area on the breast works posteriorly, then dorsally; the flanks are the last to molt. The molting process in *Neurotrichus*, once well begun, seems to be very rapid, and this may account for the sparsity of material of this genus showing molt (p. 16).

The molts of prairie dogs have been described in considerable detail by Hollister ('16). All the species comprising the genus (*Cynomys*) with one exception, undergo two changes of pelage each year. The spring molt begins in the pectoral region and in the axillae and the entire ventral surface is renewed before there is any real molt visible above. On the dorsum, the head and shoulders with occasional irregular areas on the back are invested first, and the change proceeds posteriorly. The hair

of the tail is not renewed until shortly before the autumnal molt sets in. The winter coat first appears on the extreme posterior parts of the body and progresses in a regular definite area exactly reversing the order of the spring molt.

Certain features of the molts of prairie dogs are fairly typical of rodents in general. As regards the two annual molts the same reversal in the direction of growth is described by Allen ('94 b) in the varying hare (*Lepus americanus*), by Barrett-Hamilton ('12) in the European hares, and by Howell ('14) in *Reithrodontomys*. Furthermore, it appears to be generally characteristic of the rodents that the incoming hair first appears on the ventral surface and that the change of pelage may be well under way before it spreads to the upper parts.

On the contrary, the molts of the pocket gopher as described by Bailey ('15) are of a very exceptional character. As in many other rodents there are normally two molts each year. In the first of these, investment proceeds from the nose posteriorly, but contrary to the usual rule, it lags behind on the ventrum. In the spring, the change of pelage is accomplished by a succession of molt waves which pass over the body from the nose posteriorly. There are at least five such waves each of which effects a partial change of pelage. There is no reversal in the direction of growth, as is usually the case, in the autumnal molt. The assumption of the winter pelage is accomplished in two molt waves.

In contrast to the conditions described in birds there appear to be but few suggestions of ancestral stages in the juvenal pelages of mammals. According to Friedenthal ('11) the young Indian elephant has reddish hair similar to that of the mammoth, while in echidna, a hair coat in the young precedes the development of a coat of bristles. In this connection it may be recalled that in the pelage of young lions faint spots may be seen which have been regarded as a repetition of the leopard-like markings of their ancestors.

## 6. PELAGE PHASES IN PEROMYSCUS

1. *The juvenal pelage*

At birth the body is devoid of hair and pigment except for the vibrissae and supraorbital cilia. On the second day the upper parts begin to assume a bluish-black color and the hair may be seen coming through the skin of the pigmented area. A day or two later, the ventral white hair may be observed. In the meantime the skin of the dorsal surface has become intensely pigmented. The appearance of pigment on the ventral surface is retarded for several days. This is due to the fact that the terminal part of the hair is devoid of pigment.

At the age of four to five weeks, the young are, as a rule, in full juvenal pelage. There are no further traces of pigment in the skin, which is now flesh color. This pelage, like the later ones, is made up of a fine soft underfur and a sparser coat of much longer and coarser overhair. As is the case in the adult pelages of many other rodents, the hairs of the underfur are banded or ticked, being of a blackish plumbeous or slate color basally, with a narrow subterminal zone of pallid mouse gray (Ridgway), while the tips are black. The overhairs are not of the ticked or agouti type, lacking the subterminal band. The general effect on the dorsal surface may be described as between neutral and deep neutral gray.

The juvenal pelage of the ventral surface, like that of the dorsum, is made up of underfur and overhairs. Basally, the color is the same as in that of the dorsal surface, but the distal region is white. The lateral line of demarcation between the dorsal and ventral surfaces of the body is very sharply defined (fig. 16).

The microscopic structure of the hairs in the juvenal pelage is essentially the same as described by Sumner ('18) for the adult.

There is, however, a very evident difference in the proportionate number of the different kinds of hairs. The slender hairs with but a single axial row of pigment bodies, alternating with the air spaces, are much more numerous, while the yellow pigment is much reduced in the subterminal bands. The overhairs are

attenuated at the base, being no larger at this level than the hairs of the underfur. Both kinds of hairs are very much flattened, but the larger ones show no local attenuations such as are described by Barrett-Hamilton ('16) for *Mus musculus*, though this appearance may be simulated by torsion.

The range of variation in the color of juvenal pelages in *gambeli* is shown in table 1 in which the color analyses of ten skins

TABLE 1  
*Color analyses of ten typical juvenal pelages*

	BLACK	WHITE	ORANGE-YELLOW
Buff			
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	86.8	10.0	3.1
	87.0	9.7	3.2
	87.3	8.8	3.8
	88.1	9.0	2.8
	88.4	8.7	2.8
Average.....	87.5	9.2	3.1
Dark			
	91.7	7.7	0.5
	91.7	7.8	0.1
	92.0	7.6	0.4
	92.6	7.0	0.4
	92.7	6.9	0.3
Average.....	92.1	7.4	0.4
Maximum absolute differences.....	5.9	3.1	3.5
Average absolute differences.....	4.6	1.8	2.7

are given. The light and dark extremes are each represented by five skins. As is shown in the table the black ranges from 86.8 per cent to 92.7 per cent; the white from 6.9 per cent to 10 per cent and the orange buff from 0.3 per cent to 3.8 per cent.

*2. The postjuvenile pelage<sup>3</sup>*

The transition from the juvenal to the postjuvenile pelage usually begins at the age of four weeks and is completed about eight weeks later. The new pelage first appears on the throat near the angle of the jaw, or less frequently on the anterior surface of the forelimb along the lateral line of demarcation between the dorsal gray and the ventral white. Growth proceeds toward the median ventral line of the head and, at the same time, anteriorly under the eye and ear and posteriorly over the forelimb and shoulder. From these regions, it passes posteriorly above the ventral white to the hind limbs, at the same time creeping up toward the dorsal median line (figs. 1 to 3).

On the ventral surface, the molt is regularly completed before that of the dorsum. As shown in figure 2, growth proceeds from the throat posteriorly. In many cases, there may be no superficial indications of the change, though in some instances a definite molt line may be observed.

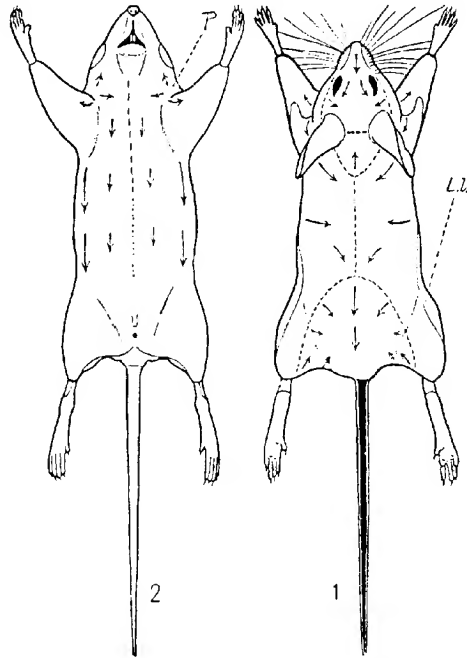
The molt may be well under way before there are any evidences of it on the surface. The details of the process can be learned only by parting the new hair as it comes through the skin. On the surface the new postjuvenile pelage is usually first seen upon the forelimbs, and somewhat later as triangular areas on the sides. These lateral areas gradually become confluent, first, as a rule, just posterior to the shoulders (figs. 17 and 18).

After this 'saddle phase' (fig. 18) has been reached, further growth for days or weeks may be limited to the region posterior to the saddle. The direction of growth is posterior and, at the same time, upward on the hind limbs from the lateral line, the region **above** the base of the tail being the last to undergo the change (figs. 19 and 20).

The molt is now completed over the whole body surface, except the region extending on the dorsal surface from the tip of the snout to the shoulders. The investment of this area may occur soon after that of the rump, but usually only after an inactive

<sup>3</sup>This account of the postjuvenile pelage has been given in a former paper (Collins, '18).

period which in extreme cases may be as long as two months. In this region the postjuvenile pelage first appears anteriorly on the tip of the snout, passing posteriorly to the eyes, thence as two diverging strips to the anterior insertions of the ears, the inter-



Figs. 1 and 2 Diagrams of the dorsal and ventral surfaces, showing directions of growth in the postjuvenile molt of *T. maniculatus gambeli*. The regions on which molt proceeds more or less independently are shown by the dotted lines. The longer arrows indicate more rapid growth. *L.L.*, lateral line; *p.*, point of origin.

vening space being filled in by lateral and posterior growth (fig. 1). Posteriorly, the molt line moves from the shoulders forward toward the ears, where the two areas coalesce. Growth in the two directions may occur simultaneously or that of one region may be slightly in advance of the others.

The postjuvenile pelage is somewhat longer and coarser, though still shorter than that of the adult, which it closely resembles in color. The general color effect is quite different from that of the juvenal. It may be described as varying from Saccardo's umber to sepia, with the dorsal median stripe more or less strongly marked with black. This difference in the general color of the two pelages appears to be due mainly to the increased amount of yellow pigment in the subterminal bands of the postjuvenile hairs.

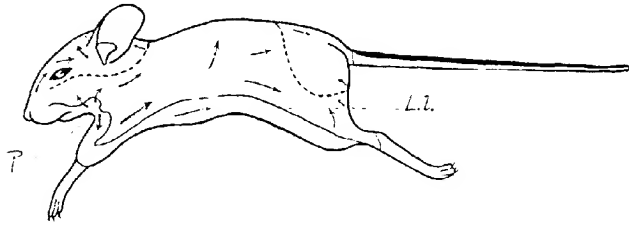


Fig. 3 Diagram of lateral surface, showing directions of growth in the postjuvenile molt of *P. maniculatus gambeli*. Symbols as in figures 1 and 2.

As will be seen by a comparison of the color analyses given in tables 1 and 2, the postjuvenile pelage is somewhat lighter than the juvenal, but the difference in the appearance of the two pelages is due mainly to the large increase in the percentage of orange-yellow in proportion to white.

The postjuvenile molt of two other races of California deer-mice (*P. m. sonoriensis* and *rubidus*) as well as in a 'yellow' mutant of *gambeli*, which appeared about five years ago in the murarium stock is essentially the same as above described.

In the assumption of the postjuvenile pelage the first coat seemingly is wholly replaced, but the replacement is actually only partial. This condition was first discovered in *P. californicus insignis* and in *P. eremicus fraterculus*. In young specimens which have just assumed the postjuvenile pelage, by parting the hair, a second subterminal band was found, slightly proximal to, fainter, and more grayish in color than the one distal to it. It was observed furthermore that this band disappeared as the

adult pelage came in, being present ahead of the advancing line of incoming pelage and absent back of it. In these species the postjuvenile hair is somewhat longer than that of the first coat and the extra subterminal band is that of the persistent juvenile hair.

In *P. m. gambeli*, the difference in the length of the juvenile and postjuvenile hair is not often sufficient to separate the two

TABLE 2  
*Color analyses of ten typical postjuvenile pelages*

	BLACK	WHITE	ORANGE-YELLOW
Buff			
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	80.9	6.4	12.6
	81.1	7.4	11.4
	81.2	7.5	11.3
	82.0	9.4	8.5
	82.5	7.4	10.1
Average.....	81.5	7.6	11.0
Dark			
	81.1	9.4	3.4
	87.0	9.8	3.2
	87.5	10.0	2.4
	87.7	9.5	2.8
	88.8	7.8	3.3
Average.....	86.4	9.3	3.0
Maximum absolute differences.....	7.9	3.6	10.2
Average absolute differences.....	4.9	1.7	8.0

subterminal bands. In this subspecies, however, the presence of juvenile hair in the postjuvenile pelage was demonstrated in the following manner. The terminal portion of the juvenile hairs including the subterminal bands was clipped off shortly before the postjuvenile molt. About two weeks after the molt was completed, samples of the pelage were plucked out and subjected to microscopical examination. The persistence of a part of the juvenile pelage was shown by the presence of numerous truncated hairs.

The foregoing account of the assumption of the postjuvenal pelage is based primarily upon a study of living cage-born *Peromyscus*. These observations have been supplemented by observations on a considerable number of juvenal mice trapped from time and upon an examination of a total of nearly three hundred juvenal skins in the collection of the Museum of Vertebrate Zoölogy, representing the subspecies *sonoriensis*, *rubidus* and *gambeli*.

Since Sumner ('18) has found that *Peromyscus* are modified, in some cases to a marked extent, by captivity, it would be but natural to suppose that the process of molt and the appearance of the pelage might also be modified. It is rather surprising to find, however, that, with few exceptions, such is not the case. In the worst cases of deformity there may be some evidences of disturbance but on the other hand, the postjuvenal molt and the appearance of the pelage are, in most instances, normal even when the mouse is quite obviously abnormal in other ways.

### 3. *The adult pelage*

In order to determine the manner and more particularly, the age at which the adult pelage is acquired, a series of fourteen postjuvenal *gambeli* were kept under observation and examined at approximately weekly intervals for a period of eleven months.

Of the fourteen individuals in this series, one began to assume the adult pelage at the age of  $2\frac{1}{2}$  months, while in a second instance there was no sign of the adult pelage until  $4\frac{1}{2}$  months after the date of birth. These two individuals represent the extreme cases. The average age, however, is slightly more than three months.

The points of origin and directions of growth are much the same as in the assumption of the postjuvenal pelage, although the process is less regular. The advance of this molt wave is almost invariably marked by a molt line which frequently does not appear on the surface until the molt has reached the middorsal region. The incoming pelage is slightly longer, the black markings may be somewhat coarser and the buff tints are more pronounced. Not infrequently this first molt wave appears on the

anterior part of the body before the juvenal pelage has been replaced on the top of the head but more usually there is an inactive period of one or two weeks following the completion of the postjuvenal molt. A complete change of pelage is not effected by this first molt wave. The major portion of the adult pelage appears to be acquired at this time while the irregular partial molts which follow seem to complete the change. Occasionally these partial molts show something of the normal sequence and direction of growth. That these supplementary partial molts do not modify the color of the pelage is shown by the fact that, although the new hair appears in isolated patches, the pelage never presents a spotted appearance. Furthermore, the molt lines which in rare cases accompany this second molt wave are always faint. It seems quite certain that normal individuals of this subspecies undergo no appreciable change in color after the age of  $6\frac{1}{2}$  to 7 months.

As brought out by the color analyses in tables 2 and 5, the differences in the appearance of the postjuvenal and adult pelages are due chiefly to the increase in the percentage of orange-yellow in the latter. The assumption of the adult pelage in the above series was begun during the autumnal molting season. Some disturbance of the process because of this fact might well be anticipated. That this is not the case was shown by the study of a second series of twelve individuals which began to assume the adult pelage during the months of January, February, and April. The molts of these mice were essentially the same as in the first series. In seven of the eight cases which were followed through the first autumnal molting season, there was a marked increase in molting during this time and, in some instances, definite molt lines appeared. Their ages at this time varied from nine to fourteen months.

#### 7. SEASONAL CHANGES IN PELAGE

This discussion of seasonal changes in the pelage of adult *Peromyscus* is based upon a study of the pelage changes observed in a series of thirteen adult *gambeli* which were trapped in old, worn pelage in the vicinity of the Scripps Institution, September 13

to 15, 1918. These mice were kept under observation in the murarium for a period of eleven months. During the autumnal molting season, they were etherized and examined for evidences of molt, at approximately weekly intervals. Of this stock eleven individuals survived nine months, when through accident the number was reduced to eight.

The average condition as regards amount of pelage change is shown in figure 4. The most obvious characteristic of the seasonal molts is the absence of sharply defined molting periods. The curve does, however, show a maximum during October and November. This may be designated as the period of the autumn-

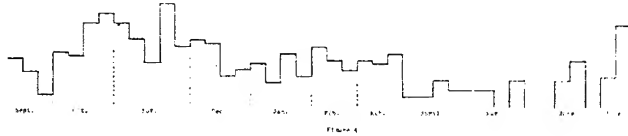


Fig. 4 Histogram showing the continuous character of molt in adult gambeli. The extent of molting was determined by grading the card records (p. 9) according to an arbitrary scale.

nal molt. As followed in individual cases, it is much more pronounced than is indicated by the curve which is based on the average condition of all the members of the series.

While the time of the first appearance of this molt is quite variable, it is most often found beginning in October. As regards points of origin and directions of growth, it is much less regular in character than those involved in the assumption of the postjuvenile and adult pelages. Despite numerous irregularities in the process, such that the molts of no two individuals are alike, the same general plan as that of the postjuvenile molt is followed.

The new pelage first appears, as a rule, on the anterior aspect of the forelimb or on the throat, often near or at the point of the jaw. From these points of origin, the molt wave passes posteriorly over the ventral surface, up under the ears and posteriorly over the shoulders, at the same time creeping up on the sides. The change on the ventral surface may proceed in advance of that on the dorsum, but not infrequently the two surfaces may undergo the molt simultaneously or the first-mentioned condi-

tion may occasionally be reversed. The dorsal surface of the head may be the last to undergo the change, as is normally the case in the assumption of the postjuvenile pelage, but this sequence is not always followed. It will be recalled that in postjuvenile mice, the incoming pelage of the head, in most instances, grows from the tip of the snout posteriorly and from the back of the neck anteriorly, the two areas coalescing in the region of the ears. This peculiar mode of growth has been observed only occasionally in autumnal molts.

The first wave of the autumnal molt does not effect a complete renewal of pelage. The greater part of the pelage seems to be replaced at this time but the change appears to be completed by a second molt wave, much more irregular than the first. The gradual advance of the first wave is, with a few exceptions, marked by a molt line which may be barely discernible or quite distinct. Occasionally, individuals are found which undergo molt without showing a molt line. This may be due to the fact that there is no difference in color in the old and the new pelages or to the almost simultaneous growth of the new pelage over the greater part of the dorsum. This first molting period is usually from four to six weeks in length and in some individuals may be even more prolonged.

The second molt wave (if for convenience, it may be so termed) may appear on the head or forelimbs before the first wave has passed over the posterior part of the body, but more typically the two waves are separated by an inactive period of two weeks or more. The second wave is rarely marked by a molt line. Not infrequently, there is some suggestion of regularity with respect to points of origin and directions of growth, but in most individuals this molt is exceedingly irregular. Small patches of incoming pelage may appear on any part of the body with no suggestion of bilateral symmetry. Oftentimes, there is no obvious break between this series of partial molts and the insidious changes of pelage which continue with but few interruptions throughout the year.

As in most of the species of *Peromyscus*, there is no spring molt, although some changes of pelage may be observed during this season such as may be found at other times as well.

Of the thirteen individuals in this series, six were females. Five of these gave birth to broods early in the molting season. In every case there was a cessation of molt for a period of about four weeks following the birth of the brood, i.e., during the nursing period. In three of the five cases the molt was well under way and in a fourth case was in its initial stages when interrupted by the appearance of the broods. Although breaks in the continuity of the process of molting occur at other times and in the records of the males as well, in view of the fact that these inactive periods are longer than usual, and that they occur in the midst of the autumnal molting season, the coincidence of the nursing periods and the interruptions of molt suggests a causal nexus. Retardation in the molts of females, evidently of a more pronounced character, are mentioned by Hollister ('16) in his discussion of the molts of *Cynomys*. According to his account, "Breeding females are always slow to acquire the fresh coat, renewal being greatly retarded by lack of excess vitality" (p. 9). A similar delay in the molting of breeding females has been observed by Allen ('94 b) in many of the *Sciuridae* especially in the genera *Sciurus* and *Tamias*.

That the seasonal molts are essentially the same in captivity as in nature is shown by a series of nearly two hundred skins of adults, in which every month of the year is represented. As shown in figure 5, which is based upon the data given in table 3, *specimens may be found undergoing some change of pelage, any month of the year*. In most cases, however, these changes are very slight. A maximum is reached during September and October, i.e., the period of the autumnal molt. A second maximum, not very marked, however, occurs in December. This is probably due to the supplementary partial molts which follow the first molt wave.

#### 8. TYPES OF HAIR IN THE ADULT PELAGE

##### 1. *The underfur*

Like the juvenal and postjuvenal pelages, that of the adult is made up of a thick coat of fine, soft, longer and somewhat coarser overhair. The hairs composing the underfur on the dorsum are

of the banded or agouti type. The variation in length ranges from 8 to 10 mm. The width varies from about 10 to 50  $\mu$  in the broad flattened middle portion, through the greater diameter. Throughout the greater part of its length, the hair is very much flattened especially so in the region of and just below the sub-

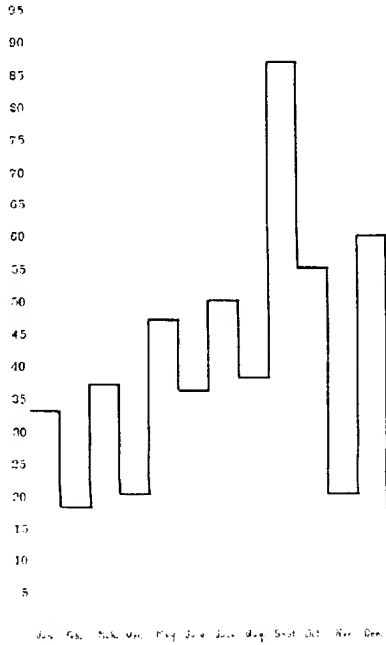


Fig. 5 The ordinates indicate the percentage of the total number of skins representing each month of the year, which show evidences of molt.

terminal band. The minute structure of the agouti type of hair is shown in figs. 22 to 28 of plate 2. When subjected to microscopical examination, three regions may be distinguished. The outer, thin transparent cuticle, composed of thin, flattened cells. Within this are the cortex in which the cell outlines are lost, and the medulla, which forms the axial portion of the hair. As shown

in these figures, the cells of the cuticle give to the surface of the hair a rough, jagged appearance which is especially well marked in the basal portion of the hair, becoming less prominent as the distal region is approached.

With regard to localization, two types of pigment may be recognized, i.e., axial and cortical. Throughout the greater portion of the length of the hair the pigment is confined to the medulla in which dense axial masses of sepia granules alternate with air spaces. It is of interest to note in this connection that the axial pigment masses adhere to the proximal walls of the air

TABLE 3

	MOLTING	NO EVIDENCE OF MOLT	TOTAL NUMBER OF SKINS	PERCENT OF SKINS SHOW- ING MOLT
January, 1917.....	6	12	18	33
February.....	3	14	17	13
March.....	7	12	19	37
April.....	4	16	20	20
May.....	7	8	15	47
June.....	8	14	22	36
July.....	10	10	20	50
August, 1916.....	5	8	13	38
September.....	14	2	16	87
October.....	6	5	11	55
November.....	1	4	5	20
December.....	6	4	10	60
Total number skins in series.....			186	

spaces. This peculiar detail of structure is especially well shown in figure 22. This is a very constant condition and it is possible while studying a hair under the microscope, to distinguish distal and proximal regions of a segment in this manner alone, where, as in figures 26 and 27, the cuticular striae are not readily seen. The individual granules are oval in form. Below the subterminal band they appear fairly uniform in size but in the apical region they are much smaller.

As shown by the figures, the cortex is devoid of pigment below the level of the subterminal yellow band. First appearing as sparse scattered granules at the level where the yellow axial

pigment is being replaced by the sepia, the pigment granules in the cortex become more numerous toward the tip of the hair, nearly or quite concealing the axial pigment masses. These granules are found to be arranged in longitudinal striae. In the attenuated terminal region of the hair the axial pigment disappears and with it, apparently, the medulla as well. The extreme tips are pigmentless.

In the proximal portion of the hair (fig. 22), there is but a single row of pigment masses alternating with the air spaces, but, in the middle region (figs. 23, 24, 25) the number of rows may vary from one to four, decreasing again to a single row in the subapical region.

The broad hairs having from two to four rows of pigment masses greatly outnumber those with but a single row. In the latter type of hair, the axial masses of sepia granules do not as a rule, continue distal to the subterminal yellow band.

The yellow pigment shows only a slight tendency to assume a granular form, and occurs mainly in flocculent or amorphous masses. The transition from the sepia to the yellow of the subterminal band is a gradual one. As the band is approached from below, the sepia pigment masses become less dense, and the granules fewer and less compacted together. Somewhat farther along minute barely discernible masses of yellow are found with a few scattered sepia granules embedded within them. The yellow gradually increases in density and in richness of color, while the sepia granules become fewer and finally disappear. Distally the same gradual transition occurs, the yellow being replaced by the sepia.

There are no intermediate stages between yellow and sepia. In the regions where both occur, each is distinct. On the other hand, the yellow varies from pale lemon to a rich orange in the more granular portions. This variation appears to be due merely to differences in the density of the flocculent masses.

Despite the fact that three pigments (i.e., black, brown or chocolate, and yellow) have been described in the hair of *Mus musculus* by Miss Durham (Bateson, '03), I am led to conclude, with Wright ('17) and with Sumner ('18), that there are but two

pigments present, viz., a dark sepia brown and yellow. There is an unbroken series of gradations from the sepia brown granules of the cortex in the subapical region to the almost dead black granules which compose the dense axial pigment masses. Throughout the series, the approach toward black appears to be proportionate to the size of the granules, and of the density of the clusters in which they occur.

The underfur of the ventral surface is made up of hairs which are the same in structure as the agouti hairs of the dorsum with the difference that the distal region is devoid of pigment. The transition from the pigmentless to the heavily pigmented condition is a gradual one. There are at first a few scattered granules in each segment; these gradually increase in number through twenty-five to thirty segments when the maximum is reached.

Along the lateral line of demarcation between the agouti fur of the dorsum and the ventral white, various transitional stages in the reduction of pigment may be found. The axial pigment immediately below the subterminal band is the first to disappear. The subsequent reduction appears to proceed in both directions and from the tip of the hair as well, the axial yellow being the last to disappear.

## *2. The overhairs*

The main structural features of the overhairs are shown in figures 29 to 34 of plate 3. They are not of the agouti type, and there is but one type of pigment evident, the sepia. Basally, the overhairs are identical in structure and size with the agouti hairs described above. One important difference in the localization of pigment is to be noticed. In the agouti type of hair, the cortical pigment rarely extends below the level of the subterminal band. In the overhairs, on the contrary, the cortical pigment always reaches a much lower level and may extend almost to the base. In this proximal region, the cortical granules appear to be identical in size, form and color with those making up the clusters in the medulla. In the distal portion of the hair, however, the granules in the cortex become very minute and increase greatly in number, becoming so numerous as to

conceal the axial pigment. As in the agouti hair, these minute granules are arranged in streaks or striae extending lengthwise.

As the cortical granules become larger, in passing from the apical region to the base, the sepia color grows darker, passing by insensible gradations into what appears to be black. The medial flattened portion of the overhairs is typically somewhat broader than in the hairs of the underfur. The overhairs are 10 to 13 mm. in length, and the maximum width through the greatest diameter ranges from 40 to 60 $\mu$ .

The overhairs of the ventral surface (figs. 46 and 47) are identical in structure, including the form and arrangement of air spaces with the corresponding hairs on the dorsum, but pigmentless throughout the greater part of their length.

In a few species of the genus, the hair of the ventral surface is entirely pigmentless and in rare cases this condition may be found in species in which the basal part of the hair is normally pigmented. One such instance has been observed by Sumner (MS.) in *P. m. rubidus* and I have found a few *P. m. gambeli* which showed this condition in the juvenal pelage, returning to the normal condition in the later pelages. An unusual variation in the opposite direction was called to my attention by Mr. Harry S. Swarth, of the Museum of Vertebrate Zoology. In a specimen of *P. m. hylaeus* trapped by him in Alaska the hair of the ventral surface shows practically no reduction in pigmentation though the tips are more buffy than those in the typical agouti hair of the dorsal surface.

### 3. Caudal hairs

One of the most striking characters of the species *maniculatus* is the sharply bicolor tail. There is a very dark, dorsal median stripe extending from the base to the extreme tip. Outside this stripe, the hair is pigmentless to the base.

The hair of the tail is found to be quite unlike the body hairs in form and structure. As is shown in figures 35 to 41 of plate 4, the surface of the hair is much smoother, the cuticular striae being scarcely visible. As compared with the agouti hairs, they are very much shorter and larger in diameter and the tips are relatively blunt.

The average length is about 2 mm. and the greatest diameter is approximately  $50\mu$ . The medulla is segmented, but there is never more than a single row of pigment bodies though these bodies are much larger than those found in the body hairs. The cortex is very heavily pigmented throughout almost the entire length of the hair, the pigmentation being most intense in the subapical region. There are various degrees of pigmentation from a very slight amount found in the cortex alone to an intense concentration both in cortex and medulla.

The caudal hairs are not of the banded or agouti type. Sepia brown which appears black in dense masses is the only pigment present. However, one finds, in rare instances, a hair which suggests the agouti type. In one individual, a few hairs were found in which the axial sepia pigment was confined to the proximal part of the hair, being replaced by a trace of yellow pigment in the middle region. In such hairs, the cortical pigment is likewise aborted at this level.

The pigmentless caudal hairs appear not to differ from those of the dorsal median stripe save in total absence of pigment.

#### 4. *Vibrissae*

The vibrissae are by far the largest hairs which occur on the body. In length the variation is from 10 to 40 mm., while the largest diameter ranges from 100 to  $140\mu$ . Unlike the hairs previously described, which are slightly spindle shaped, the vibrissae are the largest at the base. As in the hairs of the tail, the cuticular striae are very much reduced. There is but little evidence of segmentation in the medulla. The degree of pigmentation varies from a mere trace at the level of the lateral line of demarcation to the intense condition shown in figure 43 of plate 5. The striated appearance due to the linear arrangement of the minute sepia granules is very marked. No trace of the yellow pigment is found to occur in the vibrissae. The cortex, unlike that of the caudal hairs does not appear homogeneous, but shows rather faint longitudinal striations which appear to be air spaces (figs. 42, 44, 45). Unlike the rather blunt caudal

hairs, the vibrissae have very long slender tapering points. This apical pigmentless portion of the hair (fig. 45) is composed of cortex and cuticle alone.

#### 5. *Supraorbital cilia*

The supraorbital cilia, of which there are but two on each side, occur immediately above the eye. Next to the vibrissae these cilia (including the postorbital cilia as well) are the most conspicuous hairs on the body. The posterior cilium is about 15 to 20 mm. in length, while the second which is slightly anterior to the first is only about two-thirds as long. This difference in length is a constant character.

Slightly posterior to and below the eye is found another similar hair—the postorbital cilium—which is about the same in length as the more posterior of the supraorbital cilia.

As in the vibrissae, most of the pigment occurs in the cortex. The cuticular striae are a little more prominent and the medullary cylinder shows more of a tendency to become segmented.

#### 6. *Hair of the ears*

Several types of hairs are peculiar to the external ear. One of these occurs on the outer surface near the anterior margin. These hairs are similar to those of the tail stripe but differ from them in some respects. The cortical pigment, which is arranged in striae, is less dense, and the medullary cylinder is segmented in a very regular fashion. There is but a single row of axial pigment bodies, which adhere very regularly to the proximal wall of the air spaces. A second type of hair found in the same region is quite unlike any other hair found on the body. These hairs are much longer than those just described and have no medulla. The pigmentation of the cortex is less intense, while the cuticular ridges are much more pronounced than in any of the types of hairs thus far described with the exception of the body hairs. These hairs, it may be added, are relatively sparse. Most of the hairs found on the inner surface are without pigment. The medullary cylinder is very regularly segmented as in the similar pigmented hair of the outer surface.

In mammals, it appears to be generally true that exposed areas of the skin tend to become pigmented, whereas regions protected from the light remain relatively free from pigment. This relation between depth of pigmentation and exposure to light is well seen in the ear. The basal portion is covered with short agouti hairs. Here the skin is unpigmented. The distal part of the ear which is almost hairless is heavily pigmented.

#### *7. Hair of the feet*

The ectal and parts of the plantar surfaces are covered with short closely appressed hairs which are entirely pigmentless. *They are about the same in diameter as the agouti hairs, but are less than 1 mm. in length.* Most of these hairs appear to be identical in structure with the distal, pigmentless zone of the underfur on the ventrum. Some of them, however, resemble more nearly the pigmentless hairs of the tail.

The most conspicuous hairs on the manus are found in small tufts on the outer surface of the wrist. They are of about the same length as the agouti hairs but quite different in structure. The medulla is very much reduced or absent and the cortex is marked with dim striations such as those seen in the vibrissae.

The theory has been advanced by Friedenthal ('11) and others that in the phylogenetic development of the hair coat in mammals the so-called sinus hairs (vibrissae, supra-, and postorbital cilia, etc.) were the first to appear. There are a number of facts for which the theory offers a plausible explanation. As already noted in the description of the juvenal pelage, the vibrissae and supraorbital cilia are the only hairs present at birth. Furthermore, after a comparative study of a large number of mammals, Friedenthal found that in the almost hairless forms, the sinus hairs are persistent throughout. It may be further noted that throughout the various orders of mammals, the vibrissae have undergone relatively little differentiation as compared to the diversity in the structure of the body hairs.

As figured by Friedenthal, the body hairs of a young anteater (*Manis*) are very similar to the distal part of the vibrissae in *Peromyscus*. There is no medullary cylinder, and the cuticular

striae are barely perceptible. In the tanrek (*Centetes ecaudatus*) an insectivore from Madagascar, the medulla is present and very similar in form to that in the vibrissae of *Peromyscus*, although relatively somewhat smaller. The body hair of *Echidna* is without a medulla, but in the disposition of the cortical pigment and in the relatively smooth cuticle looks very much like the vibrissae. The same striking similarity is seen in the body hair of the Kangaroo and *Ornithorhynchus*.

If we may regard the hairs of the lowest mammals, in general as approaching most nearly to the primitive condition, then it may be said that, in structure, the vibrissae, supra-, and postorbital cilia and the hairs on the ear and feet, in which the medulla is reduced or absent, are the most primitive hairs on the body.

#### 9. SPECIFIC DIFFERENCES IN MOLT

In a few groups of mammals, the molts of allied genera have been found to differ to some degree. Reference has already been made to Jackson's description of generic differences in the molts of moles (see p. 13). Nelson ('09) finds that American rabbits may be separated into two classes by peculiarities of molting: 1) those which have but one annual molt and, 2) those undergoing a vernal as well as an autumnal change of pelage. In some instances, the species in the two groups belong to different subgenera of the same genus. Osgood ('09) has found some indications of a similar condition in *Peromyscus*. Mice of this genus in general undergo but one annual change of pelage. However, in the case of *P. melanotis*, which is found at high altitudes in the mountains of central Mexico, there are apparently two annual molts.

The fairly definite manner in which the postjuvenile pelage is assumed in *P. m. gambeli* suggested the possibility of the existence of specific differences as regards points of origin and directions of growth of the incoming pelage. Accordingly, the molts of several other species were investigated.

As shown in figure 9, in *P. eremicus fraterculus*, the postjuvenile pelage first appears on the surface of the skin, on the median ventral surface slightly posterior to the forelimbs. From

these centers, growth proceeds peripherally, and the greater part of the ventrum is covered before the molt wave reaches the dorsum. In *gambeli*, it will be recalled, the postjuvenile pelage first appears to surface view on the forelimbs. In *eremicus*, on the other hand, it is first seen between the mid-points of the sides and the hind limbs, and the change may be well under way before the forelimbs are invested. Otherwise, the process is much the same as in *gambeli*.

In *P. californicus insignis* (figs. 7 and 8) the postjuvenile pelage first appears in the axillae and on the forelimbs. The incoming hair appears almost or quite simultaneously in the two regions. Aside from the differences as regards points of origin the molt is essentially the same as in *P. eremicus fraterculus*.

The points of origin of the postjuvenile pelage of *Mus musculus* are shown in figure 6. In other respects the molt is very similar to that of the species of *Peromyscus* above described.

No differences have been found in the postjuvenile molts of the three subspecies, *sonoriensis*, *rubidus*, and *gambeli*, all of which occur within the state of California. However, in the subspecies *austerus* which inhabits the Puget Sound region the process appears to be somewhat different from that in the California forms.

In the series of more than two hundred skins representing this subspecies in the Museum of Vertebrate Zoology, there are fifty-eight which show various stages in the transition from the juvenile to the postjuvenile pelage.

Of these skins, five show early stages of the process. In all of these, the molt is obviously unlike the corresponding stages in *gambeli*. It is evident from these skins that the new pelage does not appear first on the forelimbs or at the point of the jaw as in the California races. While in all cases the molts are too far advanced to show the initial stages, it is clear that the points of origin are on the posterior ventral surface. Molt may be well under way before the incoming hair appears on the throat and forelimbs—the first regions to undergo molt in *gambeli*. Another difference which appears to be characteristic is the position of

the region on the dorsal median line where the lateral areas of postjuvenile pelage coalesce to form the saddle (fig. 18). This is farther posterior than in *gambeli*.

If these few skins may be taken as indicating the typical condition in *austerus*, we have, then, a case of differentiation in the mode of molt in subspecies widely separated geographically.

#### 10. REGENERATION

The effect of the cutting of hair upon its subsequent growth has been studied by Remesow ('93) and Bischoff ('98). Rabbits and dogs were used by Remesow in his experiments. His method was to remove the distal, medial, and proximal third of the hairs on selected areas on successive days. Five or six days later, the skin of the operated region was extirpated, stained and sectioned. Skin from the corresponding area on the opposite side of the body was used as a control. The cells of the roots of the clipped hairs were found to be larger and there was a significant increase in the number of mitotic figures. Remesow concluded, therefore, that the clipping of hair resulted in increased growth.

Bischoff repeated the experiments of Remesow, using precisely the same methods and the same animals, but with negative results. Bischoff regards the discrepancy in results as due to Remesow's failure to count the mitotic figures in a sufficiently large number of hair roots.

My own studies of the regeneration of hair, which I have described in a former paper ('18) have been confined exclusively to *Peromyscus*. Restoration was found to occur both when patches of hair were removed by clipping close to the skin and by plucking out. As in new-born mice, the appearance of the new growth of hair is preceded by an intense darkening of the skin. This intense pigmentation of the skin is shown in figures 48 to 51.

In the case of juvenile mice, it was found that the normal mode of assumption of the postjuvenile pelage could be profoundly modified by the artificial induction of regenerative processes. In these experiments, a total of about forty *gambeli* were used

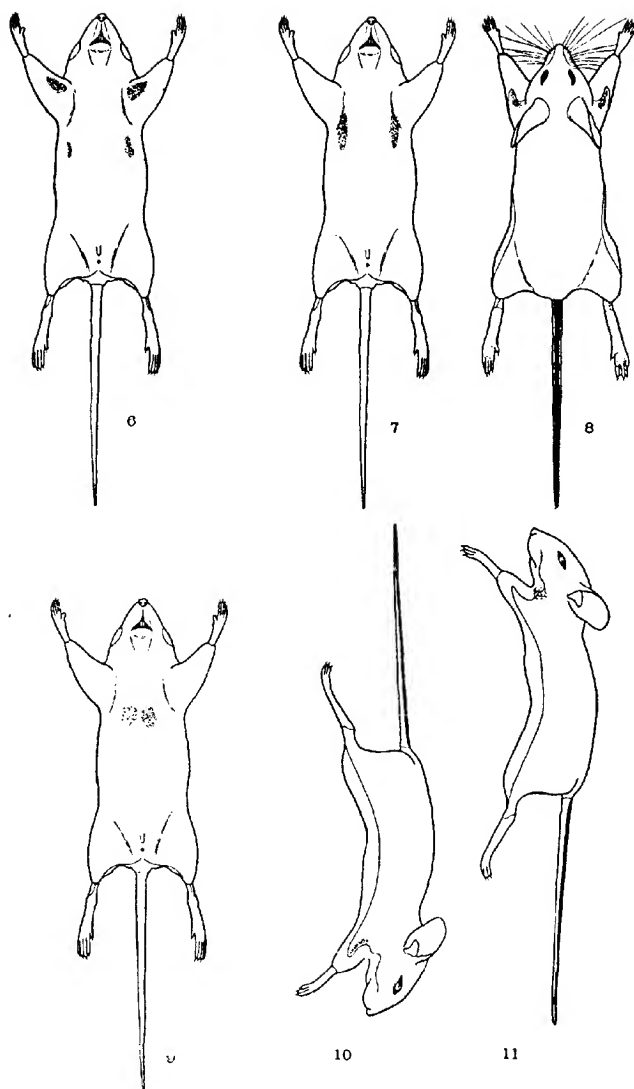


Fig. 6 Diagram, showing by the stippled areas, the points of origin of the postjuvenile pelage in *Mus musculus*.

Figs. 7 and 8 Diagrams showing points of origin (stippled areas) of the postjuvenile pelage in *P. californicus insignis*.

Fig. 9 Diagram showing points of origin of postjuvenile pelage in *P. eremicus fraterculus*.

Figs. 10 and 11 Diagrams showing points of origin of postjuvenile pelage in *P. m. gambeli*.

varying in age from two and one-half to seven weeks. The mice were etherized and the pelage removed by plucking out with the fingers. The hair is quite loose, especially just previous to a molt and may be quite readily removed in this manner without injury to the skin. In most cases the following regions were operated on:

1. The median dorsal region of the head from the tip of the snout to the base of the skull.
2. The hips and thighs.
3. About 1 sq.cm. between and anterior to the forelimbs.
4. About 1 sq.cm. in the midventral region, just anterior to the hind limbs.

If replacement on the depilated areas were to follow the normal sequence, the order would be as follows: 1) throat; 2) posterior ventral area; 3) dorsal lumbar region; 4) dorsal head region. Moreover, as already noted,<sup>4</sup> the dorsal head region is normally invested some weeks after the appearance of the new pelage on the throat and forelimbs.

Instead of following this order, restoration on the head preceded that on the hips and hind limbs—an inversion of the natural order. Growth was found to be almost simultaneous on the two denuded areas of the ventral surface, while the pelage of the hips was the last to be replaced. Without exception, removal of the juvenal pelage is followed, not by a new growth of juvenal hair, but by the precocious appearance of postjuvenal pelage on the depilated regions.

A similar instance of the premature appearance of hair characteristic of a later pelage has been described by Schultz ('15) in the Himalayan rabbit. In this animal which is a pink-eyed albino with black feet, muzzle and ears, the black markings do not appear in the juvenal pelage. By plucking out the hair on one ear, Schultz obtained animals in which one ear was black, while the other remained white until the next pelage was assumed.

In order to study the details of normal molt in adult mice, the old pelage was removed by clipping close to the skin. In all,

<sup>4</sup> See page 59 above.

seventeen adult gambeli were included in this series. The mode of replacement was found to be so irregular that the primary purpose of the experiment was not attained. Nevertheless, as a study of regeneration the experiment seemed worth while.

Despite various irregularities and individual differences, there were some suggestions of the normal process. Replacement occurred first, as a rule, on the throat and forelimbs. The investment of the ventral surface preceded that on the dorsum, while in general the molt proceeded from before backward.

The most obvious departure from the normal process was the appearance of more or less numerous small isolated patches of hair on the posterior half of the body. These 'hair islands' which appeared simultaneously with the new pelage on the throat and forelimbs, were usually more in evidence on the dorsal surface.

After the new hair on these 'islands' and on the forelimbs and throat had attained its full length there were no further evidences of regeneration for a period varying in different individuals from one to four months. Then incoming hair appeared simultaneously on all of the bare spots between the 'islands' except on the rump where, in most cases, replacement had not occurred when the animals were last examined, five months after the operation. The appearance of 'hair islands' was observed by Schultz in the course of his studies of regeneration of hair in rabbits. This investigator found after shaving large patches on the dorsal and ventral surfaces of an adult black and tan rabbit that restoration was accomplished quickly on the ventral surface while the operated area on the dorsum, with the exception of a few small patches remained bare for a year after the operation.

The individual differences which I have observed in my material in the period of time between the removal of hair and the first evidences of its regeneration as indicated by the darkening of the skin suggests a possible explanation of Bischoff's failure to confirm Remesow's results. In repeating Remesow's experiments Bischoff used, in one case, a single dog and in the second, three dogs of different ages, one cat and one pig. In view of the small number of animals used, it appears quite probable that, as an accident of individual variation, Bischoff's material was

somewhat slower than that of Remesow in responding to the conditions of the experiment. This would make possible the discordant results obtained by the two investigators.

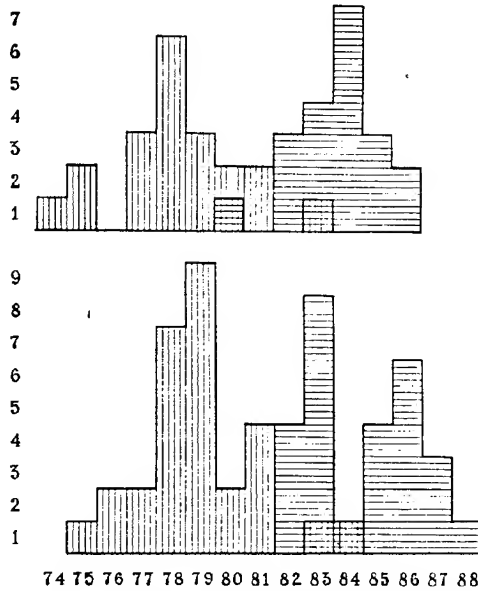


Fig. 12 (above) Histograms showing the frequency distributions of the percentage of black in the pelage of the parent series of the buff and dark extremes in *P. m. gambeli*. Vertically shaded areas represent the buff animals, horizontally shaded areas, the dark animals. Abscissas denote percentages of black; the ordinates denote the number of individuals.

(Below) Percentage of black in the pelages of the buff and dark offspring. Shading as before.

## II. THE NATURE OF COLOR VARIATIONS WITHIN A SUBSPECIES

The marked variability of mice of the genus *Peromyscus* not only as regards color, but with respect to other characters as well, is apparent from the fact that Osgood ('09) lists about forty distinguishable geographic races of the species whose differences are of such magnitude as to warrant the description of many of them as distinct species, were it not that they are connected in

all cases by intermediate forms. Furthermore, in the words of Sumner ('17): "these subspecies themselves are far from being elementary. They are composite groups, comprising, in many cases, a number—perhaps a great number—of distinguishable local types."

One of the initial steps in attacking the problem of the significance of subspecific differentiation is to determine whether the differences in question are somatic or germinal. In a discussion of 'ontogenetic species,' President Jordan ('05) some years ago called attention to the need of distinguishing between those species and subspecies whose differential characters are impressed upon each generation anew by the action of environmental agencies during the life time of an individual, and those groups whose characters are inherited. For it is obvious that those factors of the environmental complex whose effects upon the organism are limited to modifications of individual development are not concerned in the origin of species.

The transplantation experiments of Sumner ('15) involving several geographic races of the species *maniculatus* have shown that the differences which characterize these races are not somatic but heritable in character. Subsequent researches (Sumner, '18) have shown that not only are the larger differences, including color, which distinguish the main races heritable, but the same is true of certain lesser differences which distinguish narrowly localized subraces. Furthermore, certain individual differences, e.g., tail length and width of tail-stripe, within a race have been found to be to a high degree heritable.

My own studies of individual differences within a subspecies have been limited, almost exclusively, to variations in the color of the pelage in the subspecies *gambeli*. The frequent occurrence of sporadic local color variations, especially prevalent in western representatives of the genus, quite naturally suggests that these differences are due to environmental causes and produced in one or at most in a few generations.

The range of variation in color within the subspecies *gambeli* is shown in figures 54 and 57 of plate 7. By subjecting a series of skins representing the two extremes to color analysis, it is

possible to express these differences quantitatively. The variations in the various color components are shown by means of the histograms in figures 12, 13, and 14. These figures show the buff and dark extremes in a series of over four hundred specimens taken in the vicinity of La Jolla, each extreme being represented

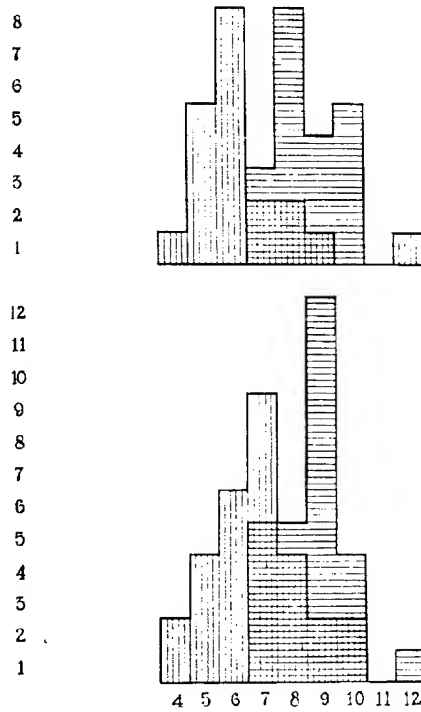


Fig. 13 (above) Variation in percentage of white in the buff and dark parents.  
(Below) Variation in percentage of white in the buff and dark offspring.

by twenty individuals in adult pelage. When the two series of skins are compared, the differences in color are quite obvious. In fact, if the buff and dark extremes of *La Jolla gambeli* were found in nature inhabiting different regions, they would undoubtedly be regarded as belonging to different subspecies.

As shown by the histograms, the greatest difference is in the percentage of orange-yellow, while the difference in the percentage of black is almost as great. Although the histograms in the case of white show a great deal of overlapping, the curve is evidently bimodal and the difference in the means is probably significant.

In order to determine to what extent these differences in color were due to fading and abrasion, the following experiment was

TABLE 4  
*Color analyses of fresh and worn pelages on opposite sides of the body*

SERIAL NO.	PERCENT OF BLACK			PERCENT OF WHITE			PERCENT OF ORANGE-YELLOW		
	Fresh	Worn	Difference	Fresh	Worn	Difference	Fresh	Worn	Difference
94	79.2	78.5	0.7	10.5	8.8	1.7	10.3	12.6	-2.3
102	84.2	80.4	3.8	9.9	10.4	-0.5	5.8	9.1	-3.3
102	82.1	80.3	1.8	11.1	9.7	1.4	6.8	9.9	-3.1
101	80.5	80.4	0.1	11.9	11.4	0.5	7.5	8.2	-0.7
93	80.7	81.6	-0.9	13.1	11.2	1.9	6.1	7.2	-1.1
111	78.9	79.4	-0.5	12.9	12.1	0.8	8.1	8.4	-0.3
89	78.1	79.0	-0.9	13.0	10.8	2.2	8.8	10.1	-1.3
109	81.5	84.2	-2.7	11.0	9.4	1.6	7.5	6.3	1.2
88	80.7	78.9	1.8	11.7	11.1	0.6	7.1	9.9	-2.4
110	80.1	80.0	0.1	12.6	10.9	1.7	7.2	9.0	-1.8
Average...	80.6	80.3	0.3	11.8	10.6	1.2	7.8	9.1	-1.5

performed. A series of about twenty adults were trapped shortly before the period of the autumnal molt. Taking advantage of the discovery that the removal of hair is promptly followed by its restoration, the pelage from the dorsal median line to the ventral white on one side of the body only was removed. Within a month, in most cases, restoration on the depilated area was complete. It was thus possible to compare old and new pelages on opposite sides of the body of the same individual and to determine directly the color differences due to fading and wear. These skins were put through the process of color analysis and the differences stated quantitatively. As shown in table 4, these differences are very small compared to those shown in the histograms in figures 12, 13, and 14.

That the color differences in question are not due, as has been suggested, to differences in habitat is shown by the persistence of the buff and dark extremes of variation without diminution in cultures reared in captivity under identical conditions. In a few cases mice of buff and of dark ancestry have been followed through four generations, and have been found to retain their differential characters.

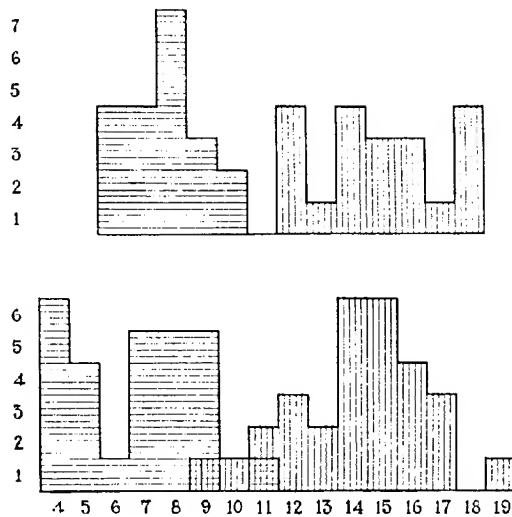


Fig. 14 (above) Variation in percentage of orange-yellow in the parent series.  
(Below) Variation in percentage of orange-yellow in the offspring.

It has been supposed by some students of the group that the buff condition represents the very old individuals in contrast to the supposed normal adults. In agreement with Osgood ('09), I find this to be erroneous. Several years ago a series of ten cage-born gambeli were set aside from the general murarium stock and designated as the 'old-age culture.' It was the purpose to determine the length of life and to ascertain something of the changes incidental to old age. After a lapse of five years six of the original ten survived. This series showed no evidence

of passing from the dark to the buff extreme with advancing age.<sup>5</sup> This conclusion is substantiated by similar observations on larger numbers of adults varying in age from two to three years.

Having determined with some degree of exactness the extent to which coloration is affected by fading and abrasion, old age, and local differences in habitat, it is observed that the extreme differences under consideration remain unaccounted for. The conclusion that these differences, which cannot be ascribed to the factors previously considered, are germinal is, I think, quite obvious. That this is true was demonstrated by the following experiment.

From a series of more than four hundred gambeli trapped in the vicinity of the laboratory under fairly uniform conditions in nature, twenty individuals representing each of the two extremes in the range of color variations were selected as parents and bred inter se. As is shown by the histograms in figures 12 to 14, inclusive, the color differences in the two parent lots were found to persist in their offspring. In each of these figures the upper and lower histograms represent parents and offspring, respectively. The vertically shaded (buff) young are the offspring of the buff members of the parent series, the horizontally shaded (dark) young being the offspring of dark parents.

The significance of the differences of the means in the parent and offspring series has been determined with respect to each of the color components, by computing their probable errors, using the formula:

$$\sqrt{(\text{probable error of first mean})^2 + (\text{probable error of the second mean})^2}$$

where the probable error of the mean is equal to:

$$\frac{\pm 0.6745 \times \text{standard deviation}}{\sqrt{\text{number of terms in series.}}}$$

The means, their differences, and the probable errors are given in table 5. In every case the differences are found to be statistically significant. Even in the percentage of white in the

<sup>5</sup> The last survivor of this group died at the age of five years, eight months.

buff and dark offspring where the histograms show a great deal of overlapping, the difference of the means is eight times the probable error.

As the result of crossing the buff and dark extremes, a series of forty-three offspring was obtained. When examined in the adult pelage these  $F_1$  mice were found to be intermediate in coat color, though a few individuals closely approached the condition of the buff and dark parents. The  $F_2$  generation comprised a series of thirty-five animals, some of which, however,

TABLE 3

	BLACK	WHITE	ORANGE-YELLOW
Mean percentages			
Buff parents (20).....	78.25 $\pm$ 0.32	6.40 $\pm$ 0.19	14.90 $\pm$ 0.32
Dark parents (20).....	83.65 $\pm$ 0.21	8.55 $\pm$ 0.15	7.75 $\pm$ 0.18
Buff offspring (29).....	78.90 $\pm$ 0.24	6.79 $\pm$ 0.19	14.17 $\pm$ 0.28
Dark offspring (27).....	84.48 $\pm$ 0.23	8.70 $\pm$ 0.15	6.70 $\pm$ 0.26
Differences of the means			
Parent series.....	5.40 $\pm$ 0.38	2.15 $\pm$ 0.24	7.15 $\pm$ 0.37
Offspring series.....	5.58 $\pm$ 0.33	1.91 $\pm$ 0.24	7.47 $\pm$ 0.38

were not in full adult pelage when the experiment was brought to a close by my leaving La Jolla. When the freshly killed animals of the  $F_1$  and  $F_2$  lots were compared in the flesh, the range in color appeared to be about the same in each.

It is quite obvious that the so-called 'buff' and 'dark' phases are not inherited in simple Mendelian fashion as are the color mutations described by Sumner ('18) in *Peromyscus* and as the color phases in some of the birds, the screech owls, for example, appear to be. On the other hand, the genetic behavior of these intra-racial differences, like that of the similar differences which distinguish the various races is of that type which is regarded by some of our present day biologists as 'blended inheritance,' in a non-Mendelian sense, by others as a complex case of Mendelian inheritance in which multiple factors are involved.

Similarly, the color differences within the desert race, *sonoriensis*, which is described by Osgood ('09) as somewhat dichro-

matic, appear to be heritable. A few individuals representing the light and dark extremes were selected and mated. The light culture proved to be sterile, but from the dark mating, twenty offspring were obtained. These were compared with the skins of the light and dark parents, and were found to belong distinctly to the latter category. As regards the subspecies *rubidus*, Sumner (MS) has a record of an exceptionally dark pair whose young were also dark.

In view of these facts, I am inclined to the view that throughout the genus as a whole the differences in color described as dichromatic and which have lead to the description of 'pale' and 'dark,' or 'buff' and 'gray' phases are mainly genetic in nature.

This conclusion is somewhat at variance with the view that mice of this genus are very plastic as regards coloration and responsive to slight changes in the environmental complex—a view which appears to be fully warranted when we recall the numerous sporadic variations often of an extremely local character which are encountered in nature. Nevertheless, there are certain facts not yet considered which show that coloration within a subspecies may remain unmodified even in the face of marked changes in the environment. Although Osgood ('09) says of *gambeli* that "Specimens slightly darker or lighter than the average may be found almost anywhere in the range, as the animal seems to respond to local environment very readily" (p. 70), nevertheless he finds that "In spite of this frequent variability *gambeli* is not a respecter of zones, as appears in many localities, notably on Mount Shasta where it ranges unchanged from the base of the mountain to the rocky cliffs above timberline" (p. 70).

The desert race, *sonoriensis*, seems likewise to be no respecter of zones. In a series of seventy-six skins in the collection of the Museum of Vertebrate Zoology, collected in localities ranging in altitude from -178 ft. in Death Valley to 11,600 ft. in the White Mountains, I find no significant differences in color. In fact, there appear to be no greater differences in this series as a whole than are to be found in a series of similar size taken in one locality in the Mohave Desert.

Perhaps the most striking instance of the relative stability of these color differences is to be found in some of the mice reared in captivity. The structural modifications exhibited by cage-born mice are described by Sumner ('18) as follows:

"It was early found that the cage-born mice depart from the wild type in certain rather striking respects. They are, on the average, considerably smaller than the latter, and have tails, feet and ears which are shorter not only absolutely but relatively. In extreme cases these malformations may fitly be termed deformities" (p. 291). Now in view of the fact that color is frequently the most obvious, and in some instances practically the only criterion by means of which geographic races may be distinguished, it is surprising to find that this character appears to be as a rule only slightly or not at all affected by the conditions of captivity.

As pointed out by Sumner ('18), certain regions of the skin are more or less heavily pigmented, though the skin is devoid of pigment over the greater part of the surface of the body. The regions showing the presence of pigment most clearly are the ears, the tip of the snout, plantar surfaces of the feet, and the scrotum in males. The pigmentation of these areas is most intense in *rubidus*, the darkest race, and least marked in *sonoriensis*, the palest of the contrasted races. By comparing the average grade of foot pigmentation in the paler and darker halves of series of *gambeli* and *sonoriensis*, Sumner ('18) found the averages for the darker halves to be slightly greater. The differences were so small, however, as to be of doubtful significance. My buff and dark series of *gambeli* afforded more favorable material for testing this point, for here only the extremes of variation in coat color were utilized. As is shown by the histograms in figure 15 there is no evidence of a correlation between the two pigmental characters under consideration. While it is true that the mean grade of the dark series is slightly greater, the difference of the means is less than twice the probable error and may, therefore, be regarded as non-significant.

If these intra-racial color differences and certain morphological differences, as well, are not attributable to the moulding

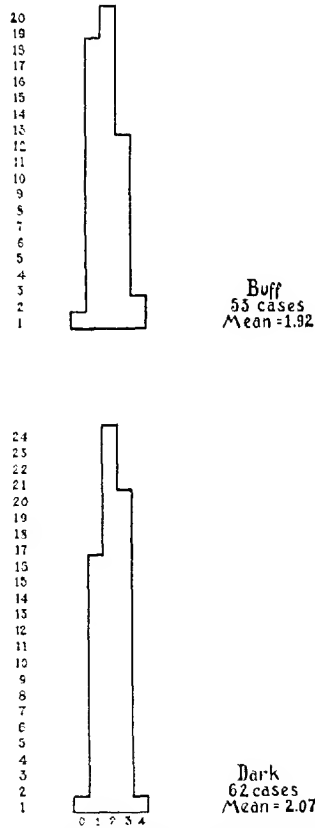


Fig. 15 Histograms showing the frequency distribution of foot pigmentation in buff and dark gambeli.

influence of environmental impacts upon plastic living material, then how are we to account for those peculiarities of distribution which seem to indicate so clearly a susceptibility to slight changes in external conditions? What of the origin of these minute heritable variations? This is the fundamental problem of evolution, the solution of which apparently becomes more remote with each advance in this field of investigation.

## 12. SUMMARY

1. During the life-cycle of mice of the genus *Peromyscus*, three pelages are assumed, namely, the juvenal, the postjuvenal, and the adult.

2. The assumption of the postjuvenal pelage occurs in a fairly precise and definite manner somewhat comparable to molt in birds. There are definite points of origin and definite directions of growth. This process has been found to differ in different species. The later molts are much more irregular in character.

3. Seven types of hair, characteristic of different parts of the body make up the adult pelage. These hairs are found to differ in microscopical structure.

4. The formation of pigment and the growth of hair may be brought about, irrespective of season, by the removal of hair on any part of the body. The regular process of molt may be profoundly modified in the case of molts thus artificially induced.

5. As a rule, changes in color due to fading and abrasion are slight. The color differences observed within a subspecies and described as 'buff' or 'light' and 'dark' or 'gray' phases cannot be ascribed to seasonal changes through which any individual may pass during the year.

6. These color phases are not due to environmental differences, acting during the lifetime of a single individual or even within a few generations. Individuals representing both extreme phases have been taken in the same locality. Furthermore, their descendants, reared in captivity under identical environmental conditions for several generations, have bred true to type.

7. The buff or light phases do not, as has been suggested, represent old as contrasted with younger adults. Specimens kept in captivity for a period of nearly six years have not undergone any such change.

8. The skins used in the study of color variations were subjected to color analysis and the differences have been treated quantitatively.

9. Pigmentation of the feet was found to vary independently of the color of the pelage.

10. The differences in color are mainly genetic in character. The offspring of buff and dark parents are likewise buff and dark and, moreover, these differences are apparent soon after birth. These color phases are not alternative in inheritance. When the buff and dark strains are crossed, the offspring are of an intermediate character. The  $F_2$  generation is also intermediate in coloration. These results may with equal facility be regarded as due to 'blending inheritance,' in the original sense, or to the action of multiple factors.

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## PLATE 1

### EXPLANATION OF FIGURES

Figs. 16 to 21 show various stages in the postjuvénal molt of *P. maniculatus gambeli*.

16—Young in full juvenal pelage.

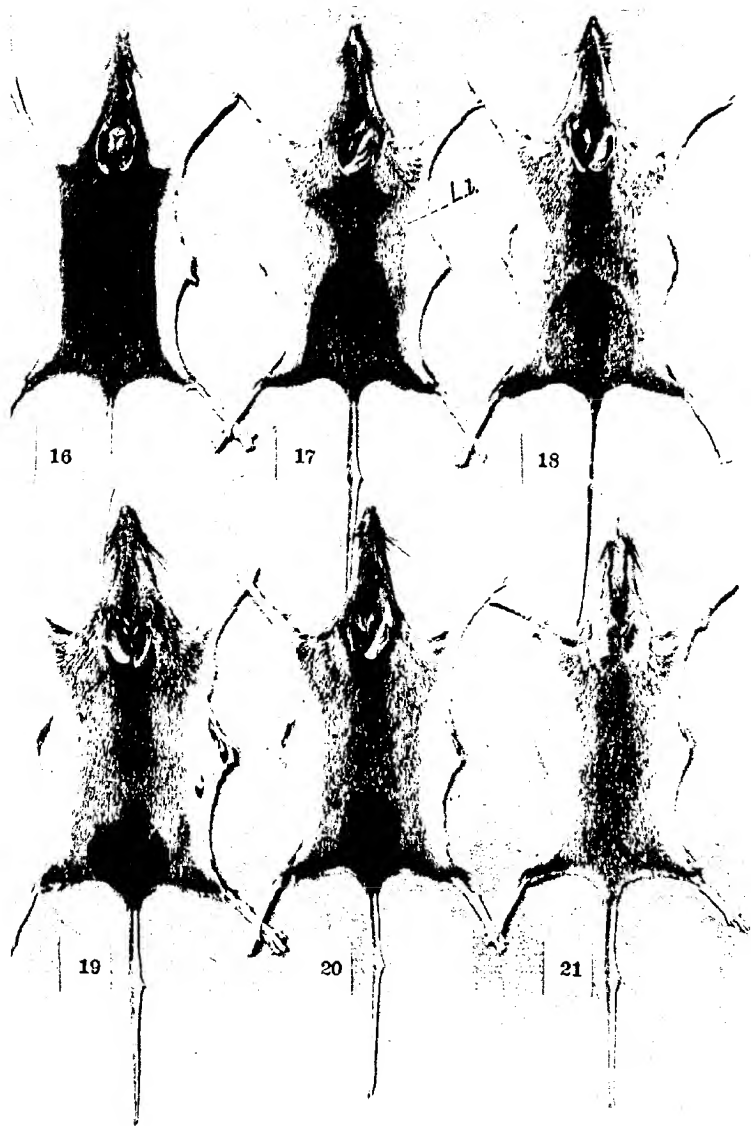
17—Showing the growth of the postjuvénal pelage from the lateral line (*L.L.*) upward.

18—Early 'saddle phase.' The lateral areas, as seen in figure 17, have become confluent on the dorsal median line.

19—Late 'saddle phase,' in which the juvenal pelage still persists on the head and back of the neck, and on the rump. The dark strip extending from the shoulders posteriorly to the rump is the dorsal median stripe of the post-juvénal pelage. The difference between this stripe and the juvenal pelage on the rump is not well shown in the figure, though the actual colors are quite unlike.

20—A later phase, showing further decrease in size of the areas of juvenal pelage on the head and rump.

21—Full post-juvénal pelage.

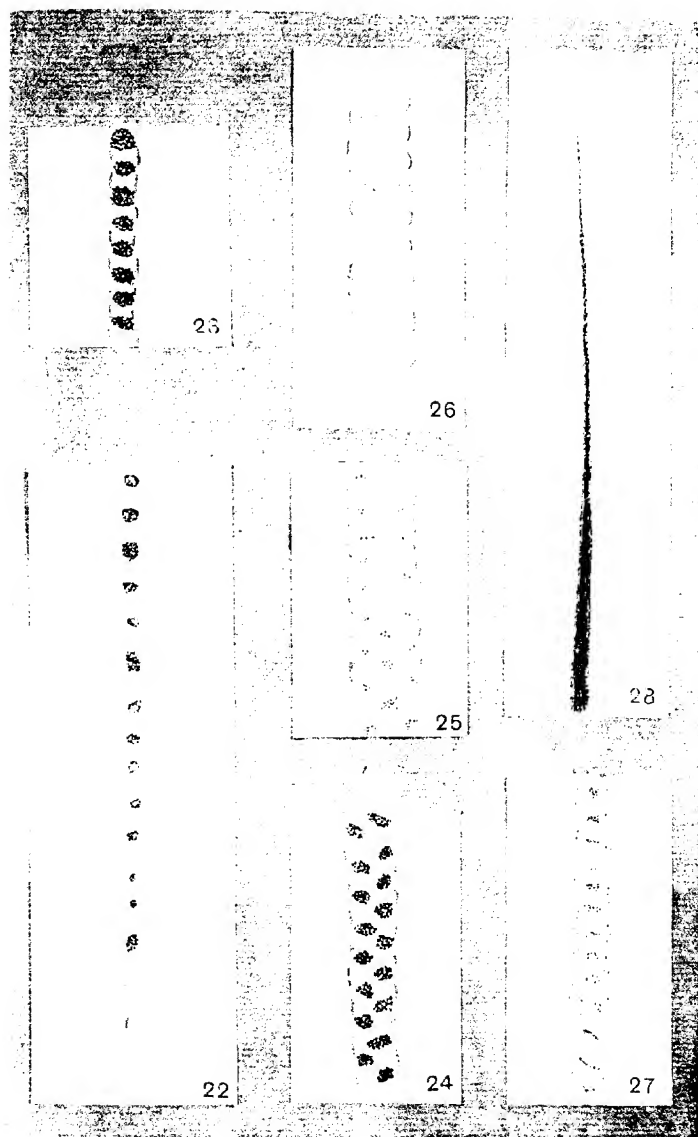


## PLATE 2

### EXPLANATION OF FIGURES

All figures showing the structure of hair drawn with camera lucida. All drawings  $\times 470$ . Figures 22 to 28 show the interconical structure of the banded or agouti type of hair at different levels from the proximal region (fig. 22) to the tip (fig. 28).

Note the variation in the diameter from the basal region to the tip; also the gradual decrease in the size of the cells of the cuticle and the manner in which the masses of pigment granules adhere to the walls of the air spaces.



## PLATE 3

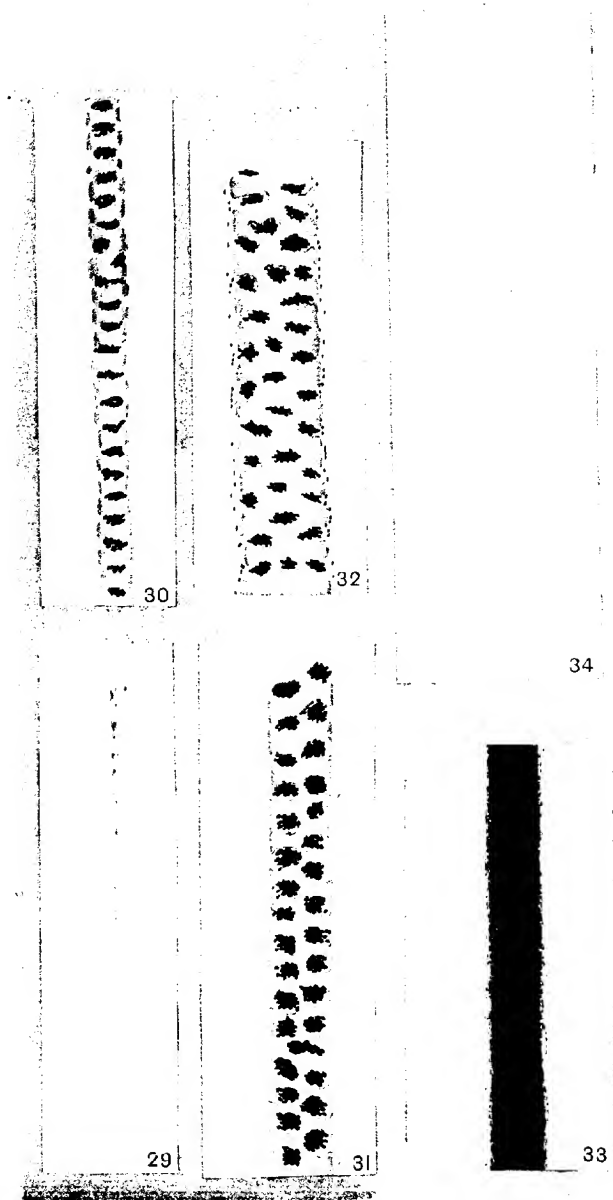
### EXPLANATION OF FIGURES

29 and 30 show the structure of the basal portion of an overcoat from the dorsal surface.

31 and 32 represent segments of the middle region. Note the presence of cortical pigment granules in figure 32, and their absence in the corresponding level of the agouti hair (fig. 24).

33 Subapical region. The cortical pigment is very dense and may entirely conceal the axial pigment masses. Note the striated arrangement of the cortical granules.

34 Pigmentless tip.



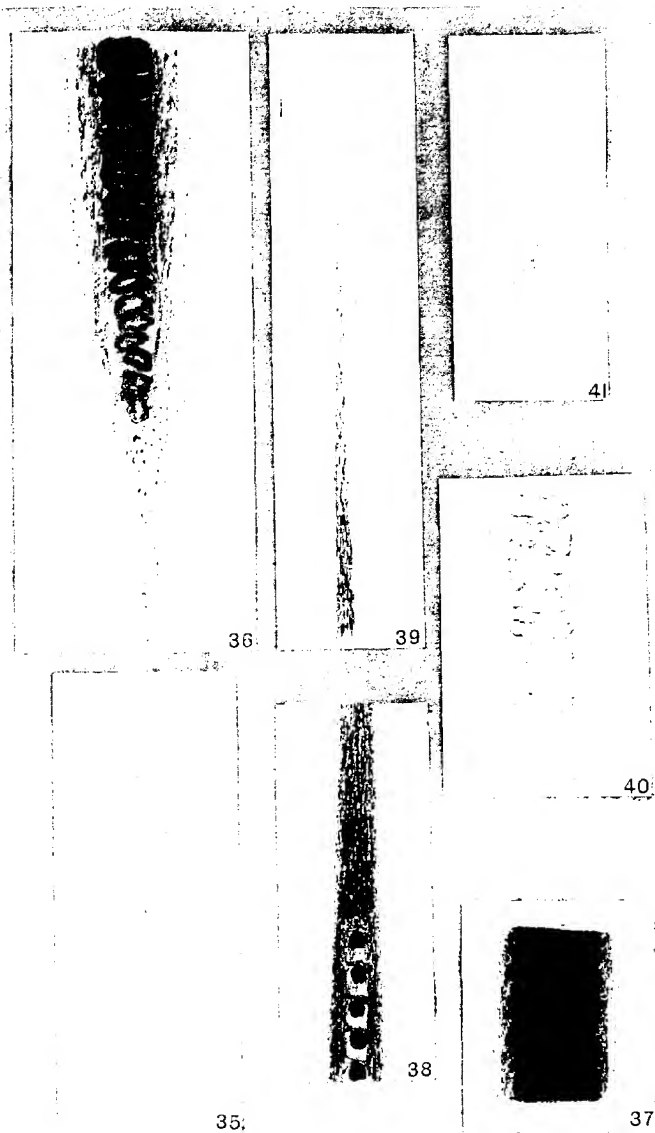
#### PLATE 4

##### EXPLANATION OF FIGURES

35 to 39, inclusive, show the structure of a pigmented hair from the dorsal tail stripe.

In comparison with the agouti hairs and overhairs, observe the extent of the cortical pigment, the smooth surface of the cuticle, and the difference in the structure of the medulla.

40 and 41 show the structure of the medial and distal portions of a pigmentless hair from the tail.



## PLATE 5

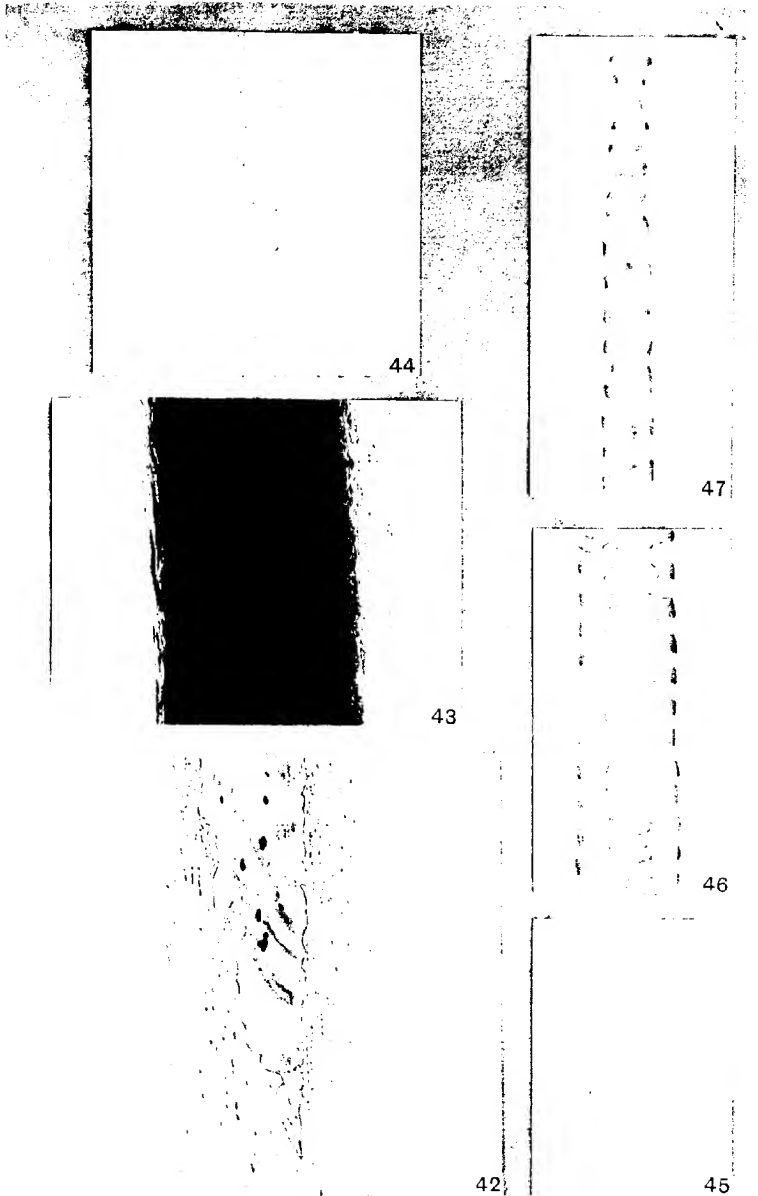
### EXPLANATION OF FIGURES

42, 43, 44, and 45 show the structure of a vibrissa at proximal, medial and distal levels.

Note the intense pigmentation of the cortex in the middle region and the striated arrangement of the granules; also observe the absence of prominent cuticular striae such as occur on the agouti hairs.

46 Segment of middle region of an overhair from the ventral surface.

47 Segment of ventral overhair distal to the preceding.



# PLATE 6

## EXPLANATION OF FIGURES

- 48 and 49. Unilateral pigmentation of skin in response to depilation.
- 50 and 51. Normal bilaterally symmetrical pigment patterns, as they occur in the assumption of the postjuvénal pelage.

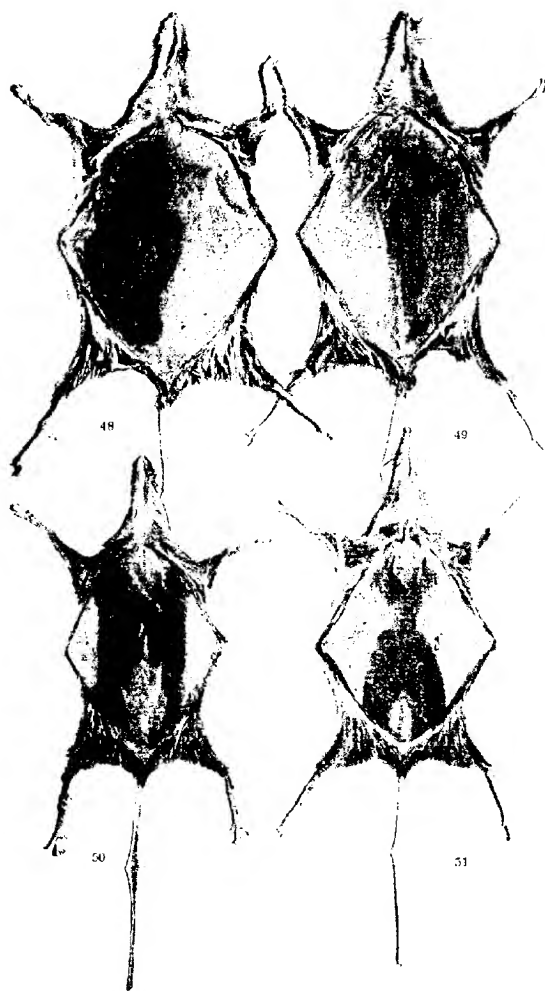


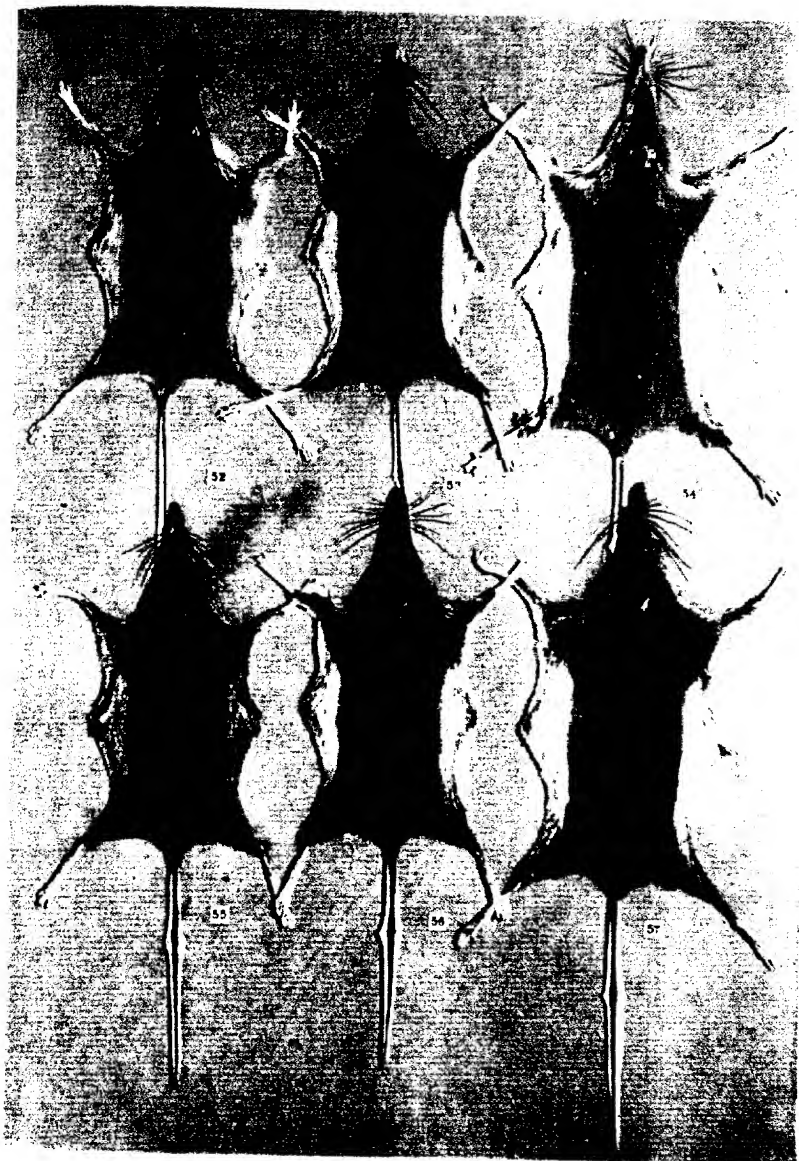
PLATE 7

EXPLANATION OF FIGURES

Variations in coat color in *Pan. gouldii*

- 52 Juvenal pelage in buff series.
- 53 Transition from juvenal to postjuvenal pelage in buff series.
- 54 Adult pelage—buff series.
- 55 Juvenal pelage in dark series.
- 56 Transition from juvenal to postjuvenal pelage in the dark series.
- 57 Adult pelage—dark series.

Note that the difference between the extremes of variation is seen in the juvenal as well as in the later pelages.





# PHOTIC ORIENTATION IN INSECTS WITH SPECIAL REFERENCE TO THE DRONE-FLY, *ERISTALIS* TENAX AND THE ROBBER-FLY, *ERAX RUFIBARBIS*<sup>1</sup>

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THIRTEEN FIGURES

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## INTRODUCTION

Reactions to light have probably been more extensively studied in insects than in any other group of animals, and yet, while numerous facts having a profound bearing on the problem of orientation have been established, there is practically nothing known as to precisely what reactions are involved in the process. We have theories demanding that the legs respond thus and so, but no observations demonstrating how they actually do respond. We have theories demanding certain relations between various conditions of illumination and response in the

<sup>1</sup> I am much indebted to the Marine Biological Laboratory for excellent facilities used in some of the experiments described in the following pages, to Prof. James S. Hine for generous assistance in identifying the robber-fly used, and to Prof. Wm. L. Dolley for valuable criticism.

locomotor appendages but no observations demonstrating precisely what relations obtain. For example, according to the Ray-De Candolle ('32) theory of orientation in plants as applied to unicellular forms by Verworn in 1894 and to higher animals by Bohn in 1904 and Loeb in 1906<sup>2</sup> and accepted by Garrey ('18) and others, the process of orientation depends upon the relation in the rate of activity or movement of opposite sides of the organism. In an insect which is not oriented the legs on one side, according to this view, take longer steps and consequently move faster than those on the opposite side causing it to turn just as a boat turns when the oars on one side move faster and more efficiently than those on the other; and the rate of movement on opposite sides bears a definite relation to the amount of light received by the two eyes, such that when this is equal the feet on opposite sides move at the same rate and when it is unequal at different rates.

Bohn puts the matter very precisely in the following statements ('09 a, p. 9): "The side which is more strongly stimulated by the light moves more rapidly than the other (*marchera plus vite que l'autre*), and this results in a forced turning movement."

<sup>2</sup> Garrey ('18, p. 101) implies that Loeb's first views concerning orientation ('88) are in accord with his recent views. I cannot agree with him in this. In 1888 Loeb maintained that orientation in animals is determined by the direction in which the rays penetrate the tissues and not by difference of intensity on opposite sides of the organism. He says ('88, p. 2): "Die Orientirung der Thiere gegen eine Lichtquelle wird, wie bei den Pflanzen (J. v. Sachs) bedingt durch die Richtung in welcher die Lichtstrahlen die tierischen Gewebe durchsetzen, und nicht durch die Unterschiede in der Lichtintensität auf den verschiedenen Seiten des Thieres." It is consequently evident that Loeb in 1888 was in harmony with Sachs who consistently opposed De Candolle.

In an explanatory footnote ('05, p. 2) dated 1903 he says, "It is explicitly stated in this and the following papers that if there are several sources of light of unequal intensity, the light with the strongest intensity determines the orientation and direction of motion of the animal." That is, orientation is not determined by the relation in the illumination of opposite sides of the organism in accord with De Candolle's theory ('32) as applied to animals by Verworn ('94). In 1906 Loeb, however, accepted the De Candolle-Verworn theory and applied it to higher forms. He says ('06, p. 130) that orientation in symmetrical organisms is determined by the relation in the intensity of light on the photosensitive elements on opposite sides. Thus it is evident that Loeb's first explanation of orientation differs fundamentally from his more recent explanation.

('09 b, p. 5) "Orientation (tropisme) presents itself to us as the forced result of the inequality of the *same* activities in the right and the left half of the body, an inequality which is purely quantitative, not qualitative. . . . In other words, orientation (tropisme) is not an activity (*une activité*), it is the result of an inequality between certain activities, simple or complex, which occur on one side of the body and the same activities which occur on the opposite side."

Garrey expresses similar ideas in his work on the effect of light on the tonus of the muscles in the legs of the robber-fly and its relation to orientation. He says ('18, p. 107): "The legs on the side of the illuminated eye cannot be widely separated nor can those of the other side be easily approximated, thus tending to produce a wider arc of progression on the side of the blackened eye." If there is thus, as Garrey says, a wider arc of progression on one side than on the other, it is evident that the feet on one side must move faster than those on the other side. Various statements made by Loeb in reference to the process of orientation lead to the same conclusion. He says, e.g. ('13, p. 463): "Wenn nun ein Tier seitlich vom Licht getroffen wird, so wird eine Hälfte des Nervensystems in stärkeren 'Phototonus' geraten als die andere. Wenn bei einem solchen Tiere Impulse zu einer Lokomotion stattfinden, so wirken die Impulse nicht wie gewöhnlich auf beide Seiten des Tieres in gleicher Weise, sondern die mit beiden Hirnhälften verbundenen Muskeln werden verschieden stark arbeiten." and ('18, p. 83): "Motile plant organisms like *Volvox*, are driven to the source of light, owing to differences in the tension of the contractile organs on the shaded and illuminated side, and the same is true for animals like insects." Patten also appears to have the same idea as to the method of orientation. He says ('19, p. 457): "Orientation is attained and maintained by a transmission of impulses to the muscles of locomotion which is proportional bilaterally to the excitation of the symmetrically located photo-receptors."

According to these explanations, therefore, orientation is the result of a balance between the effect of the rate or extent of movement of the locomotor appendages on opposite sides,

just as it is in a row boat which has no rudder and is propelled and guided with oars, the rate of movement in the appendages is directly proportional to the amount of light received by the receptors connected with them, the processes involved are not dependent upon the location of the stimulus in the receptors, the stimulating agent acts continuously, not intermittently, and the orienting stimulus continues after the organism is oriented just as it does during the process of orientation.

Views concerning orientation which are in all essentials in accord with these characteristics have been designated "the difference of intensity theory," "the continuous-action theory," "the tropism theory," "Loeb's tropism theory," "the Verworn theory," "the muscle tension theory," and "Loeb's muscle tension theory." I shall in the following pages refer to them as the De Candolle-Verworn or the Ray-Verworn theory or the tonus hypothesis.

The object of the experiments and observations described in the following pages was primarily to ascertain the movements involved in the process of orientation in insects and the relation between these movements and various conditions of illumination. Is orientation in insects dependent upon the relation in the rate of movement of the locomotor appendages on opposite sides as it is in *Volvox*; is it dependent upon bending of the body as it is in earthworms, *Turbellaria*, *Arenicola* and blow-fly larvae; is it dependent upon the position of a median locomotor appendage as it is in *Euglena*, tadpoles and fishes; or is it dependent upon the direction of movement of the locomotor organs as it is in some vertebrates and in automobiles? What is the relation between the movements of the locomotor appendages resulting in orientation and the location of the stimulus in the photo-receptors; how does the stimulating agent act in controlling these movements, and what is the relation in magnitude between the energy received and the response?

## MATERIAL, METHODS AND GENERAL BEHAVIOR

The drone-fly, *Eristalis tenax*, is found about flowers throughout the entire summer. Toward autumn when the nights grow cool it often comes into buildings in considerable numbers. Practically all of the specimens used in the following experiments were found in the Zoölogical Laboratory of the Johns Hopkins University. Just why these creatures come into buildings at this time of the year is not clear. It may be that this phenomenon is associated with hibernation. *Eristalis* responds to light very definitely and precisely. It is hardy, does not feign death to any marked degree, if at all, and can be kept in the laboratory in excellent condition for several weeks if it is provided with sugar, fruit juice, and a fairly humid atmosphere. It is consequently very favorable for certain work on reactions to light.

The robber-flies are usually found in dry, hot, sandy places sitting motionless, fully exposed to the sun. This habit is associated with the process of feeding. They live on other insects which are captured much as a lion captures its prey. When an insect suitable for food comes within striking distance the robber-fly suddenly darts into the air after it, seizes it with its claws and brings it to the ground. It apparently feeds only on insects that it catches itself, for it will not accept insects brought to it either dead or alive, nor will it catch insects when confined to the laboratory where it consequently lives only a few days.

Death-feigning is marked in the robber-fly. It will hold without any observable movement, for several minutes at a time, almost any position in which it is put. If, e.g., the body is pushed sidewise so as to make the insect lean strongly to one side or if the abdomen is raised so as to make the animal appear as though it were standing on its head, or if a leg is raised to an abnormal position it retains this position for considerable periods of time. This is an important point which will be referred to later. It shows that the degree of tension of muscles in the legs of the robber-fly (tonus) may be entirely independent of the immediate action of the environment.

The robber-fly is very active and agile on the wing, but very slow and sluggish on foot. On smooth surfaces, like glass, e.g., it has great difficulty in walking at all. On surfaces like ordinary paper it can walk much better, but even on these its movements are so slow and so uncertain that the observations often become tedious and exasperating. There are, however, some advantages in this slowness of movement, since it facilitates following and recording the responses.

Nearly all of the observations were made in a large dark room provided with tungsten and Nernst lamps mounted in light-tight boxes so constructed as to produce well-defined beams of light of the size and intensity desired.

Asphalt varnish was used to cover the eyes in all experiments with blinded or partially blinded specimens. To cover the eyes sufficiently to prevent stimulation by light it was found necessary to apply with considerable care, preferably under a lens, several layers of varnish. In many instances the efficiency of the covering was tested at the close of the experiment by covering both eyes and noting the effect of illumination. All records obtained from specimens in which the covering was in any way defective were discarded.

#### ORIENTATION IN NORMAL SPECIMENS ON FOOT

*Eristalis*. *Eristalis* is strongly positive in its reaction to light and it orients fairly precisely. If the direction of the rays is suddenly changed after a specimen is oriented it turns directly without trial or random movement until it is again oriented. If the direction of the rays is only slightly changed it is difficult to ascertain precisely what reactions in the legs are involved in the process of reorientation, but if the direction of the rays is changed through 90 degrees so that the light strikes the animal at right angles to the longitudinal axis it can be very clearly seen that the turning toward the light is not primarily due to a difference in the rate of locomotion, a difference in the size of the steps on opposite sides, but to a change in the direction of the movement of the feet on both sides. It can be seen that in both of the front feet there is a marked sidewise movement

toward the light, that in the two middle feet there is a similar movement but less marked while in the hind feet there appears to be no lateral movement at all. It can also be seen that in these lateral movements there is an increase in the extension of the legs on the side toward the light and an increase in flexure in those on the opposite side<sup>3</sup> (fig. 1, B). If the light is moved

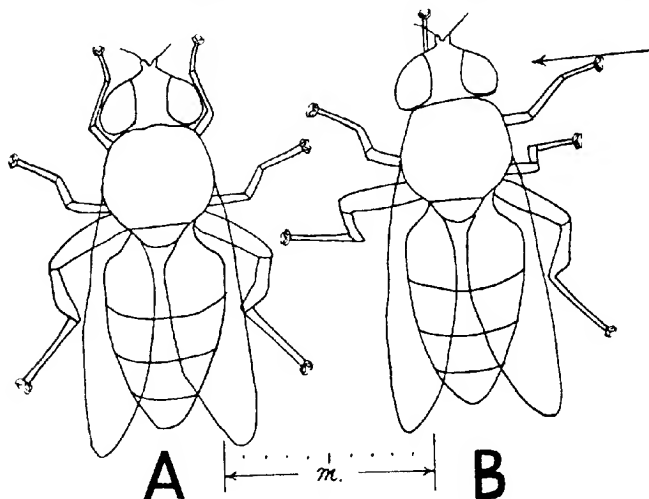


Fig. 1. A, camera outline of *Eristalis* in resting position. B, sketch indicating direction and extent of movement of the front feet toward light during process of orientation with rays at right angles to longitudinal axis. Arrow, direction of rays; *m.*, projected mm. scale.

laterally far enough so that the postero-lateral surface of the eye becomes most highly illuminated, the flies under certain conditions turn so sharply that there is no locomotion whatever, the feet on the illuminated side moving backward at the same rate as those on the shaded side move forward. In turning thus both of the front feet move laterally toward the light, while both of the hind feet move laterally from the light (fig. 2).

<sup>3</sup> Garrey ('18, p. 106) maintains that just the opposite obtains in the robber-fly, *Proctacanthus*. He contends that turning in the process of orientation and turning in cirrus movements are the same, and that in both the legs are strongly flexed on the side toward which it turns and well extended on the opposite side.

*Erax*. *Erax*, like *Eristalis*, is photopositive, but orientation in the former is not nearly so prompt and precise as it is in the latter. In the laboratory *Erax* rarely moves at all, no matter where the light is situated, unless it is touched and then it frequently merely moves the part touched, e.g., a leg or the abdomen. Moreover, without moving the feet it has a tendency to extend or flex the legs in such a way as to move the head toward the light no matter where it may be. If the light is above, the front legs are extended downward so as to raise the head; if it is below they are flexed so as to lower the head; if it is to the right both front legs are extended to the left so as to move the head to the right; and if it is to the left they are extended to the right so as to move the head to the left. In the lateral movements of the head to the right or left the entire body usually leans strongly in the direction in which the head is moved. This leaning or tilting of the body is like that observed in specimens with one eye covered. The moving of the head and the leaning of the body toward the light appears to be an attempt to orient. The head is extended toward the light as far as possible without moving the feet. This is an important point which will be referred to again in the section on the reactions of specimens with one eye covered. If the feet move and the body becomes oriented, the movements are essentially the same as they are in the process of orientation in *Eristalis*. That is they move laterally, backward or forward, depending upon the location of the source of light.

These results seem to indicate clearly that orientation in insects is dependent upon the direction of movement of the feet rather than upon the rate of movement, as Bohn et al. maintain, and that the character of the response in the legs during the process of orientation is specifically related to the location of the stimulus in the eye. It cannot be accounted for on the assumption that it is due solely to difference in the amount of light energy received by the two eyes in accord with Loeb's most recent explanation of orientation ('18). This conclusion is strongly supported by results obtained in various other observations described in the following pages.

## ORIENTATION IN NORMAL SPECIMENS ON THE WING

Both *Eristalis* and *Erax* are photopositive on the wing just as they are on foot. Detailed observations on the process of orientation were, however, made only on the former.

When *Eristalis* is set free in an ordinary room, it flies fairly directly toward the window. Orientation is, however, not very precise. This is clearly shown by the results obtained in the following experiment.

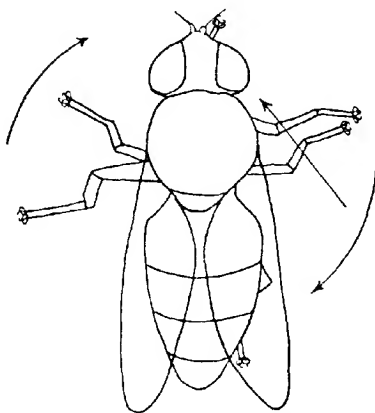


Fig. 2 Sketch of *Eristalis* indicating that when light is directed toward the postero-lateral surface of one eye, the legs on one side move forward while those on the other move backward in the processes of orientation. Straight arrow, direction of rays; curved arrows, direction of movement of feet. Compare with figure 1.

In October, 1920, a 300-watt tungsten lamp was mounted in one end of a black box 140 cm. long and 35 cm. square in cross section, and so arranged in a large dark room as to produce a well-defined horizontal beam of light directed against one of the black side-walls of the room without direct illumination of either the floor, the ceiling or the other side-walls. In this beam twenty specimens were set free, one at a time, at a distance of 8 m. from the lamp. All of the specimens flew to-

ward the light, but only one oriented accurately enough to get into the box. The rest left the beam before they reached the box, some at the right or at the left, others above or below. These results show that while orientation in *Eristalis* on the wing is not very precise, it takes place in reference to the vertical as well as the lateral plane. This conclusion is supported by the results obtained in the following observations. It is of especial interest since it has a direct bearing on the explanation of orientation as will be shown later.

Several methods were used in making observations on the process of orientation in the vertical plane. The results obtained were essentially the same in all, but they were more definite in the following than in any of the others.

Two 40-watt Mazda lamps situated 125 cm. apart on a vertical line were so arranged and screened as to produce two rather large beams of light which crossed each other in a horizontal plane passing through a point half way between the two lamps (fig. 3). The lamps were connected with a two-way switch in such a manner that when one was turned on the other was simultaneously turned off. A fly which oriented fairly accurately was then set free at a point 4 m. from either lamp, and after it had proceeded about 150 cm. toward the lighted lamp, the switch was suddenly thrown so as to turn this lamp off and the other one on, thus suddenly changing the direction of the rays vertically. In every trial when the direction of the rays was thus changed, the fly turned either directly upward or directly downward, depending upon whether the change in the direction of the rays resulted in an increase in the illumination of the upper or the lower surfaces of the two eyes.

Owing to the rapidity of movement it was not possible to ascertain precisely how the changes in direction of locomotion are related to changes in the positions of the wings; but it could be clearly seen that turning upward or downward is accomplished by changes which are the same in both wings. If, e.g., the light is directed toward the dorsal surfaces of the eyes, both wings respond in such a way as to turn the anterior end of the organism upward, and, if it is directed toward the antero-

ventral surfaces, both respond in such a way as to turn it downward. Evidently, then, the vertical turning responses in *Eristalis* on the wing must be related to a change in the location of the stimulus in the eye. It cannot be related to anything in the nature of a balancing of stimuli and responses in symmetrically located opposite organs, as demanded by the De Candolle-Verworn theory.

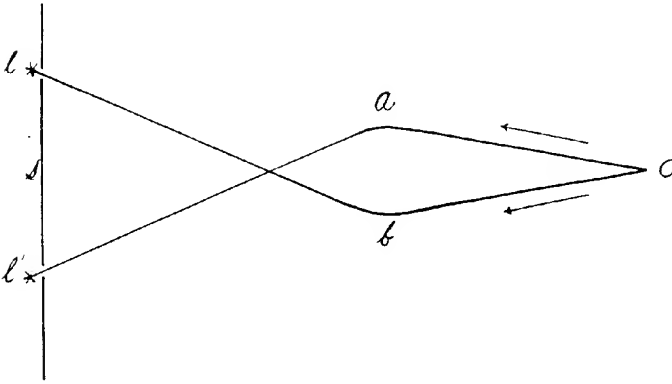


Fig. 3. Diagram showing vertical orientation of *Eristalis* on the wing. *l*, *l'*, lamps; *s*, opaque screen; *a*, point where specimens started; *a*, *b*, points where direction of rays was changed; arrows, direction of locomotion. Note that when the ray-direction was changed at (*a*) the under surface of both eyes became more highly illuminated and that when it was changed at (*b*) the upper surface became more highly illuminated. Turning was due to change in the location of the stimulus in the retina and not to unequal illumination of the two eyes, as the explanation of Bohm, Loeb and others demands.

#### REACTIONS IN INSECTS WITH ONE EYE BLINDED

It has been known for more than a century that covering one eye in insects has a profound effect on their reactions to light.<sup>4</sup>

<sup>4</sup> Garrey's statement (18, p. 106) that Loeb in 1888 discovered "the fact that photopositive flies with one eye removed, move in circles toward the good eye" does not appear to be strictly accurate. In the first place, this fact, it appears, was known as early as 1796, long before Loeb's time; and in the second place, it is not at all certain that the results obtained by Loeb in his experiments were due to the destruction of an eye. He says in reference to these experiments

Goeze in 1796 covered with an opaque varnish one eye of a hornet and found that in flying it turned continuously toward the normal eye, i.e., that it made what are commonly called circus movements. These results have been confirmed and extended to animals on foot by Treviranus ('32), Dubois ('86), Axenfeld ('99), Rádl ('03), Parker ('03), Holmes ('01, '05), Carpenter ('08), Brundin ('13), Dolley ('16), Garrey ('18), Patten ('19), Minnich ('19), and others. In most of the observations the eyes were covered but in some they were cut or burned out and in others only one was illuminated.

The results obtained by these investigators show that insects with one eye blinded usually turn, in non-directive light, toward the functional eye if they are photopositive and toward the blinded eye if they are photonegative, that contact stimulation produced by the covering on the eye tends to make them turn toward the covered eye (Dolley, '16), that in certain species in a horizontal beam of light orientation is fairly precise, that the accuracy of orientation increases with experience (Rádl, '03; Holmes, '05; Carpenter, '08; Dolley, '16; Minnich, '19), that the degree of deflection under certain conditions depends upon the intensity of the illumination, it being in positive specimens usually greater in high illumination than in low, especially in light that is somewhat diffuse (Garrey, '18; Minnich, '19), but that it may be the same or even less (Dolley, '16) and that in positive specimens a sudden decrease in illumination tends to cause sharp turning toward the covered eye while a sudden increase tends to cause sharp turning in the opposite direction (Dolley, '16).

Rádl ('03, p. 63), Bohn ('04), Loeb ('13), Garrey ('18), Minnich ('19), Buddenbrock ('19, p. 315), and others maintain that circus movements are due to difference in muscular tonus on

('05, p. 87, translation of paper published in 1888): "When one hemisphere of the brain of a house-fly is removed the same disturbances in orientation appear as after the same operation on a rabbit. The fly from which the left hemisphere has been removed moves continuously toward the right in its progressive movements." It is evident that the observed deflection to the right in the fly, may have been due to the destruction of a portion of the brain and not to the destruction of the eye.

opposite sides of the organism, and some of these authors hold that orientation is essentially the same as circus movement. Minnich, e.g., says ('19, p. 405): "The circus movement is the orienting process." Rádl and Buddenbrock contend, however, that these two phenomena are not the same. They assert that while circus movements are the result of tonus effects, orientation is brought about by responses. I did not make a detailed study of circus movements, but the results of numerous casual observations on this phenomenon in various insects, lead me to believe that, while tonus may play a part in circus movements, the great majority of such movements are much more closely related to actual responses than to tonus effects. I am of the opinion that essentially the same factors are involved in circus movement and orientation, but that tonus is normally only superficially involved in these phenomena, if at all.

The effect on the reactions of covering one eye were studied both in *Eristalis* and in *Erax*. The results obtained are in harmony with most of those obtained in other insects as presented above. The observations on *Eristalis* will be considered first.

If *Eristalis* with one eye covered receives light simultaneously from various sources, as, e.g., in front of a window, it deflects sharply and continuously toward the functional eye and consequently takes a more or less nearly circular course. In the dark room in a well-defined horizontal beam of light the tendency to deflect toward the functional eye is much reduced, especially after the covering has been on the eye for some time and the insect has been exposed a number of times. In such a beam of light the deflection is usually not continuous as it is in ordinary illumination. The specimens usually turn toward the functional eye until the longitudinal axis makes a given angle with the direction of the rays of light, and then proceed in a straight course until they reach the edge of the beam where, remaining in the light, they usually turn and follow this edge directly toward the light. The degree of deflection varies greatly with different individuals and with the same individual under different conditions. It is sometimes so small that the course is fairly directly toward the source of light.

If, after a specimen has assumed a definite axial position in a beam of light, the source is moved either to the right or to the left, it immediately turns until it again has the same axial position in reference to the rays of light. These results are, however, obtained only if the eye receives light from a single well-defined source. They are not obtained if the eye receives any considerable amount of light reflected from the surface on which the fly is located or from other objects about. The cause and the significance of this will be considered in the section of this paper entitled "Orientation in light from two sources."

The results presented above are in perfect harmony with those obtained by Carpenter ('08) on *Drosophila*, by Dolley ('16) on *Vanessa*, and by Minnich ('19) on the honey-bee. They show clearly that *Eristalis* with one eye blinded can, in response to light, turn to the right or to the left and that it can orient and move fairly directly toward the light. And since it receives light only in one eye, it is evident that these responses cannot be dependent upon the relative amount of light received by symmetrically located receptors on opposite sides of the body. The evidence indicates that they are dependent upon the location of the stimulus in the eye, and that lateral orientation, like vertical orientation on the wing, is not necessarily dependent upon symmetrical stimulation.

If, in specimens with one eye covered, the beam of light is directed toward the postero-lateral surface of the functional eye, the two front feet move laterally toward the light, the one deflecting forward and the other backward, and the two hind feet move laterally from the light, again one moving forward and the other backward, just as was observed in normal specimens with the light similarly directed toward one eye (fig. 2). The same may be said for the illumination of other regions of the eye including the anterior portions. That is, the movement of the feet induced by the illumination of any given regions of the surface of the functional eye is essentially the same in character, although perhaps not in magnitude, as it is in normal specimens when the same surface is illuminated, in spite of the fact that in these the opposite eye also receives a considerable amount

of light. If, in specimens with one eye, the beam of light is directed toward the anterior surface parallel with the longitudinal axis of the body, the feet on either side tend to move directly forward; if it is directed toward the antero-lateral surface they deflect in the direction of this surface in their forward movement; if it is directed toward the antero-median surface they deflect in the opposite direction and the animal turns toward the covered eye, the side receiving no effective illumination.

These results demonstrate conclusively that by illuminating different surfaces of either eye different reactions of the legs on both sides are induced. Illumination of the anterior surface of one eye, e.g., induces certain specific coördinated reactions in the legs on both sides; illumination of the postero-lateral surface, coördinated reactions of a very different sort. These are actual responses, not merely movements associated with difference in tonus on opposite sides. They strongly support the conclusion previously reached that orientation in insects is not necessarily dependent upon the relation in the rate of movement of the feet on opposite sides, in accord with recent explanations of Loeb, Bohn, and others, but upon the direction of movement. They indicate strongly that orientation in *Eristalis* is the result of series of reflexes dependent upon the localization of the stimulus in the eyes, differential responses to localized stimuli.

I reached similar conclusions in my study of the reactions in fire-flies as did also Buddenbrock ('19) in observation on snails and Taliaferro ('20) in observation on *Planaria maculata*. Taliaferro proved conclusively that photic stimulation of the rhabdomes at the posterior and ventral edges of the pigment-cup induces the animal to turn toward one side while stimulation of the remaining rhabdomes induces it to turn in the opposite direction.

Certain phases of these conclusions also receive considerable support from the results obtained in experiments on covering portions of the eyes in various insects by Axenfeld ('99), Rádl ('03), Holmes ('05), Dolley ('16), Garrey ('18), and others. The following reactions of *Erax* are, moreover, in harmony with them.

The orienting reactions in *Erax* are essentially the same as those in *Eristalis*. In ordinary illumination specimens with one eye covered tend to turn sharply and continuously toward the functional eye, making circus movements like those described by Garrey ('18) in a closely related form. In a beam of light in the dark room they usually take a direct course across the beam at various angles with the rays and then when they reach the edge they turn and proceed directly toward the light. If the source of light is moved to the right or to the left they turn until they are again oriented, though much more readily toward the functional eye than in the opposite direction. If the light is directed toward the postero-lateral surface of the eye, the feet on one side move forward while those on the opposite side move backward. If it is directed toward the antero-median surface of the eye and no light reaches other parts of the eye, both front feet move toward the light, and the others move in such a way as to turn the anterior end of the insect toward the blind eye. Occasionally all of the feet on both sides move laterally in the same direction so as to carry the insect sidewise toward the light, but never more than a few centimeters at a time. Sidewise movement toward the light was also repeatedly observed in *Eristalis* (fig. 4).

These reactions are not very marked and they are obtained only if the lateral and posterior surfaces of the eye receive relatively little or no light. They indicate, however, that orientation in *Erax* is, as in *Eristalis*, dependent upon the direction of movement of the feet, which is in turn dependent upon the localization of the stimulus in the eye. But how, in the experiments such as those described above, is the stimulus localized?

When light is directed toward a given surface of the eye of an insect the whole surface is more or less illuminated. How then, it may be asked, can there be anything more than a very indefinite localization? Exner ('91) and others have demonstrated that compound eyes like those found in *Eristalis* form well-defined erect images on the retina, that the image of each object centers about the ommatidium whose longitudinal axis is parallel with the rays of light received from the object and that

only those rays which are parallel with the longitudinal axis, or nearly so, reach the retina and are effective in the process of stimulation. In light then, such as was used in these experiments, a minute image of the source was formed on the retina directly beneath that portion of the surface toward which it was directed, and it is probable that the illumination of other surfaces had no effect, owing to the fact that the rays were not parallel with the longitudinal axis of the ommatidia or rhabdomes and consequently did not reach the retina. This conten-

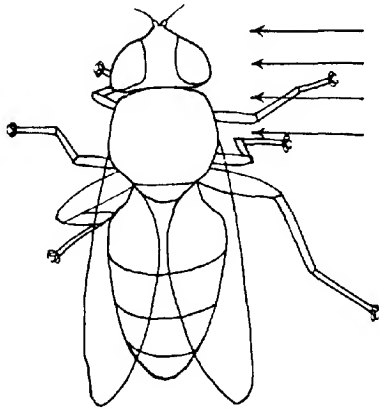


Fig. 4. Sketch showing *Eristalis* moving sidewise toward the light. Arrows, direction of rays.

tion is strongly supported by the fact that Taliaferro ('20) demonstrated experimentally in *Planaria* that only those rays which enter the eye parallel with the longitudinal axis of the rhabdomes or nearly so have any stimulating effect.

#### TILTING REACTION

It is well known that a number of different organisms in which the function of one eye has been destroyed assume abnormal postures. Holmes, referring to *Ranatra* with one eye covered, says ('05, p. 329): 'Under the stimulus of light the insect assumes a

peculiar attitude; the body leans over toward the normal side and the head is tilted over in the same direction." Similar reactions were repeatedly observed by Dolley and myself in *Vanessa* ('14-'15 unpublished) and by Garrey ('18) in a considerable number of butterflies, robber-flies, and other insects. In numerous observations on *Erax* I obtained results in harmony with those presented above.

Toads and frogs that are blind in one eye also assume an abnormal attitude but in these only the head appears to be involved and this is turned toward the blind side in place of toward the functional eye as it is in the insects. There seems to be an attempt to direct the eye more nearly forward so as to bring into the field of vision the objects directly in front of the animal (Mast, '11, p. 221).

Garrey ('18, p. 109) maintains that in some insects in which the tilting reaction does not occur when one eye is covered, it occurs if a portion of the other eye also is covered. He found, e.g., that *Eristalis* retains a normal posture if one eye is blackened but that if "one eye and the inner half of the other are blackened it shows extreme tilting of the body toward the side of the uncovered eye surface." Whether or not tilting can thus be induced in all insects which orient in light has not been ascertained.

Garrey ('18) holds that tilting in insects is the result of difference in the tonus of the muscles of the legs on opposite sides, which is in turn due to difference in the amount of light received by the two eyes, the degree of tonus of the muscles being directly proportional to the intensity of the illumination of the receptors with which they are respectively connected. He contends that the difference in tonus results in difference in the degree of extension of the legs on opposite sides and that this produces difference in the rate of movement of the feet on the two sides, resulting in circus movements and orientation. He thus maintains that circus movements and orientation are the result of tilting which in turn depends upon muscle tonus. And he asserts that this constitutes an absolute proof of the De Candolle-Verworn theory as modified by Loeb which he designates "Loeb's muscle tension theory of heliotropism."

He says ('18, p. 125): "The relation of the results of these experiments to the problem of heliotropic orientation is too obvious to require detailed discussion which could only lead to a repetition of the description of the mechanism of heliotropism which Loeb has so clearly expounded. The experiments are so completely in accordance with Loeb's muscle tension theory of heliotropism that they are tantamount to a complete proof of it."

I am not entirely clear as to what Garrey means by tonus. Tilting is undoubtedly brought about by difference in the extent of the contraction of certain muscles in the legs on opposite sides but whether this is due to phenomena similar to those which are ordinarily designated as tonus, or to phenomena similar to those which are ordinarily designated as responses to stimuli, is questionable. However, in some cases, e.g., the tilting of the head in frogs and toads, it appears clearly to be due to a response to stimuli; and it has, in my opinion, by no means been definitely established that it is not due to responses in other forms. In insects tilting is especially prominent in forms in which death-feigning is conspicuous, e.g., *Ranatra*, *Vanessa*, *Erax*, and others. None of these forms are agile on foot and the tilting in them appears to be associated with an attempt to turn toward the light without moving the feet. I have often seen normal specimens of *Erax*, when illuminated from one side, lean over strongly toward the light and hold this tilted posture without any movement of the feet whatever. The whole process of tilting in these forms appears to me to resemble responses much more closely than it does tonus effects. However this may be, the fact that in tilting the legs on one side are more flexed than those on the other, resulting in difference in the rate of movement on opposite sides and thus in turning, as Garrey maintains, shows that tilting is, at least in some cases, involved in circus movements and probably also in orientation, but the evidence presented in the following paragraphs and elsewhere indicates that it is by no means the only factor involved, that it is, indeed, a very insignificant factor. And since it has not been demonstrated that tilting is the result of

tonus, it appears that the fact that tilting may at times play a part in orientation, adds little if any support to Loeb's muscle tension feature of the De Candolle-Verworn theory, for tonus is the very essence of the structure upon which this feature rests.

1. In many insects which orient in light, *Eristalis*, e.g., there is no observable tilting when one eye is covered or when they are laterally illuminated so that one eye receives much more light than the other. Numerous detailed observations were made on *Eristalis* in reference to this, and while it was found, as previously stated, that specimens with one eye blinded make continuous circus movements in ordinary illumination, no tilting was observed in spite of the fact that the intensity of the light varied from nearly zero to direct sunlight and the fact that the turning toward the functional eye was markedly sharper in the higher than in the lower illuminations. Minnich ('19, p. 406) obtained similar results in observations on the honey-bee. In general it may be said that insects which do not tilt turn and perform circus movements quite as readily and orient quite as precisely as do those which tilt. It is consequently evident that circus movements and orientation in insects are not necessarily associated with tilting.

2. In specimens of *Erax* with one eye blinded tilting is very marked, especially if they are exposed on a white background in strong light, and such specimens, as previously stated, tend to turn continuously toward the functional eye, i.e., in the direction in which they lean. This turning is, I believe, in part dependent upon difference in the rate of movement of the feet on opposite sides, owing to difference in the extension of the legs in accord with Garrey's explanation; but only in part and this is not the only way in which the insect can turn, as Garrey appears to assume. He says ('18, p. 106): "The anterior leg of the normal side is adducted to the right, i.e., to the side of the blackened eye, and may even cross the corresponding leg of that side. The condition is a sustained, tonic one, by virtue of which the only possible movement is one in which the flexed legs pull, and the extended legs push the animal toward the

illuminated eye. The legs on the side of the illuminated eye cannot be widely separated nor can those of the other side be easily approximated, thus tending to produce a wider arc of progression on the side of the blackened eye." I have repeatedly seen *Erax*, while it was continuously leaning strongly toward the side containing the functional eye, take a straight course; I have seen it, while it was leaning from the light, go sidewise directly toward the light, all of the feet moving laterally in the same direction (similar results were obtained by Holmes in *Ranatra* ('05)); and I have seen it, while leaning in one direction, actually turn in the opposite direction, both front feet moving laterally toward the blind side, the side directed toward the light under the conditions of the experiment. That is, it turned in a direction opposite from that demanded by Garrey's tonus hypothesis. Turning in this direction was most readily obtained in specimens in which the upper half of one eye and the lower half of the other had been covered, e.g., the upper half of the right eye and the lower half of the left eye. If such specimens are placed upon a white background and illuminated horizontally from the left side, they lean to the right and turn to the left. The leaning is due largely to an increase in flexure in the proximal joints in the legs on the right side and an increase in extension in those on the opposite side. The turning is due to alternate flexure and extension in the other joints. This indicates that tilting is dependent primarily upon the illumination of the ventro-lateral surface of the eye, while turning is dependent primarily upon illumination of the lateral surface.

3. In circus movements associated with the tilting reaction the legs on the side receiving the stronger photic stimulation are more extended than those on the opposite side. In normal orientation just the reverse holds.

4. The degree of tilt in *Erax* is dependent upon the intensity as well as upon the direction of illumination; in strong light it is greater than in weak, and in any given illumination the attitude assumed is maintained for long periods of time if the intensity of the light is not changed. Garrey reached similar conclusions in his work on *Proctacanthus*. He maintains

that this shows that tonus is proportional to intensity, that light acts continuously in maintaining the tonus, resulting in the tilted posture and that this supports the continuous-action theory of orientation. The results obtained in the following observations indicate, however, that while the degree of tilt depends upon luminous intensity, the maintenance of the state of the muscles involved in the tilt is not dependent upon the continuous action of the light.

On September 23rd a specimen of *Erax* with the left eye thoroughly covered was exposed in moderately strong diffuse sunlight. The head and body at once leaned to the right fully  $45^\circ$ , the head somewhat more than the body. The posture was carefully noted and it was found that the leaning of the body was due almost entirely to difference in the degree of flexure at the proximal joints of the legs on opposite sides. The illumination was changed through various stages from total darkness to direct sunlight and from a faint ruby light to approximately 50,000 m.c. produced by a gas-filled tungsten lamp. In no case was there observed any immediate change in the degree of tilt following a change in illumination. For example, in one instance when the specimen in diffuse sunlight was leaning to the right fully  $45^\circ$  this light was suddenly replaced by faint ruby light. No observable change in the degree of leaning occurred. Three minutes later, however, it suddenly straightened up until there was no observable tilt. The ruby light was now replaced by the diffuse light. The insect remained perfectly erect. It was closely observed for six minutes and not a movement was seen, except those concerned in respiration. The paper on which the specimen was standing was now lightly tapped and it immediately leaned over to an angle of about  $45^\circ$  with the vertical. It was now exposed to direct sunlight and the paper tapped until the insect moved but no observable increase in tilt occurred.

This was repeated many times and while there was under certain conditions clearly a difference in the degree of tilt in weak and in moderately strong light, there was no observable difference in moderately strong and in very strong light. That

is, the degree of tilt seems to increase with increase in illumination only within certain limits.

These results seem to demonstrate conclusively that while the degree of contraction of the muscles in the legs of insects may depend upon the illumination of the eye, that of some being increased and that of others decreased, depending upon the location of the illumination, the maintenance of the contraction is not dependent upon continuous illumination. That is, the various muscles retain the degree of contraction induced by a given illumination for long periods after this illumination has ceased. This conclusion is, moreover, supported by the results obtained in observations on the relation between luminous intensity and the degree of flexure of the legs in normal specimens.

5. Garrey maintains that in normal specimens the tonus of the muscles in the legs of the robber-fly "*Proctacanthus*, and flies in general" is dependent upon the illumination of the eyes. He says ('18, p. 106): "In the light, normal *Proctacanthus*, and flies in general, hold the body well from the surface when walking and leave only the tracks of the feet on smoked paper, but after blackening the eyes, or in the dark, some part of the body also leaves a trail. The whole experimental picture of inactivity, muscular weakness, and incoördination, when the eyes are darkened, points to a decreased neuromuscular tonus which is normally maintained reflexly by the action of light on the eyes."

I made numerous observations on the posture of both *Eristalis* and *Erax* in natural illuminations varying from nearly zero to direct sunlight and in artificial illuminations varying from very faint ruby light to white light of more than 50,000 m.c. but was unable to observe any difference in the flexure of the legs dependent upon difference in illumination.<sup>5</sup> I did not, however, observe the effect of long exposure, and it is probable that the results obtained by Garrey were due to such exposures. However this may be, it is clear that when both eyes are equally

<sup>5</sup> Dolley obtained similar results in observations on *Vanessa* (personal communication).

illuminated, no matter how little or how much, no such marked flexures and extensions are obtained as are produced in the legs in insects in which the two eyes are unequally illuminated. The tilting reaction is therefore primarily a reaction dependent upon unequal illumination of the two eyes. It is, as previously demonstrated, dependent upon the illumination of the ventro-lateral surface of the eyes. If this surface in one eye is illuminated while that in the other eye is not illuminated, the legs on one side tend to flex while those on the other tend to extend and the insect leans toward the functional eye. If this surface is equally illuminated on both sides there is, during moderate periods of exposure at least, no appreciable change in flexure on either side regardless of the intensity of the illumination. This seems to show conclusively that the effect on flexure of the illumination of one eye is inhibited by equal illumination of the other eye. I should like to stress this point for it is of considerable importance and we shall make use of it and a number of similar inhibition phenomena in our explanation of orientation presented in the last pages of this paper.

6. In *Eristalis* or *Erax* suspended by the wings so that the feet are not in contact with the substratum, illumination and changes in illumination, either of one eye alone or of both eyes have no effect on the flexure of the legs. If one eye is covered there is no indication of greater extension in the legs on one side than on the other, such as there is when the feet are in contact with the substratum. This shows that the effect of the light on the flexure of the legs depends upon contact of the feet with the substratum, and it indicates that, if flexure is dependent upon muscle tonus, the muscle tonus is not as specifically related to the illumination of the eyes as Garrey maintains, for if it were there should be a difference in the degree of flexure in the legs on opposite sides if the two eyes are unequally illuminated and the legs ought to flex and extend in accord with changes in difference in the intensity of the illumination of the two eyes.

The results obtained in these observations seem to support the conclusion presented above, that tilting is the result of a

reaction or a series of reactions constituting an attempt to turn in the direction of greatest effective illumination, rather than the result of a specific effect of light on muscle tonus.

7. It was repeatedly observed that when *Erax* with one eye covered travels in diffuse light from a white to a black sheet of paper, it suddenly straightens up, even if it has entered a field of higher illumination, and that when it travels from a black to a white sheet it suddenly tilts, even if it has entered a field of lower illumination, i.e., it straightens up when the intensity of the light received from below decreases and it tilts when the intensity of this light increases. These changes in posture are so extensive that they can readily be seen. It was also repeatedly observed that if the lower portion of one eye and the upper portion of the other eye is covered and the insects are placed in front of a window in such a position that the eye with the lower portion covered receives more light than the other eye, they tilt toward the eye with the ventral surface uncovered. That is, under these conditions they tilt toward the eye which receives least light; whereas when one eye is entirely covered and the other is normal, they tilt toward the eye which receives most light.

These facts demonstrate that tilting depends largely upon stimulation of the ventral surface of the eye and that it is far more closely correlated with difference in the location of the stimulus in the two eyes than with difference in the amount of light received by them. It is apparently largely if not entirely independent of light received by the dorsal portion of the eye.

The facts then, that in many insects there is normally no tilting during the process of orientation; that the degree of tilt is not always immediately changed when the luminous intensity changes; that tilting is specifically correlated with the location of the stimulus in the eye; that the degree of flexure in the legs is independent of the luminous intensity when both eyes are equally illuminated; that there is in specimens with one eye covered no difference in the extension of the legs on opposite sides if the feet are not in contact with the substratum; and that certain photopositive insects, while tilting in one direction,

may take a straight course, go sidewise toward the light in the opposite direction or even turn toward the blind side, seem to show that the De Candolle-Verworn theory including Garrey's tonus hypothesis is inadequate to account for photic orientation in insects, for it is not in accord with any of these phenomena. Moreover, the results obtained in observations on the process of orientation in specimens with some of the legs on one side removed and those obtained by Dolley on the relation between the rate of locomotion and the intensity of the illumination of the eyes lead to the same conclusion. The former will be discussed in the next section, the latter in the following paragraphs.

If orientation is the result of difference in the rate of movement in the feet on opposite sides owing to difference in the tonus of the muscles in proportion with the differences in the illumination of the eyes, in accord with the De Candolle-Verworn theory as applied to insects by Loeb, Bohn, et al., then, when both eyes are equally illuminated, the rate of locomotion ought to be specifically related to the intensity.

Dolley ('17) made numerous observations on the rate of locomotion in *Vanessa* with both eyes equally illuminated in various intensities and found that it is essentially the same in all unless the difference in intensity is very great, in which case the rate is slightly greater in the weaker than in the stronger light.

It is consequently evident that if orientation in *Vanessa* is dependent upon difference in the rate of locomotion of the feet on opposite sides owing to difference in tonus, the effect of the illumination of one eye on tonus must be inhibited by equal illumination of the other eye. But if this is true, then the De Candolle-Verworn theory will not hold; for the advocates of this theory, maintain, if I understand them correctly, that the orienting factors continue to operate after the organism is oriented and the eyes are equally illuminated, as well as before. They hold, in other words, that the oriented organism is held upon its course by the continuous action of the orienting factors.

## REACTIONS IN INSECTS WITH SOME OF THE LEGS REMOVED

The advocates of the De Candolle-Verworn theory of orientation differ considerably as to precisely what factors are involved in orientation but they all appear to hold that bilateral symmetry in reference to the action of the receptors and the motor apparatus is fundamental. They maintain that orientation is the result of a balance between the effect of the action of the locomotor appendages on opposite sides, that when this effect is unequal the animals turn and when it is equal they do not turn. Loeb says ('12, p. 39): "When two retinæ (or other points of symmetry) are illuminated with unequal intensity, chemical processes, also of unequal intensity, take place in the two optic nerves (or in the sensory nerves of the two illuminated points). This inequality of chemical processes passes from the sensory to the motor nerves and eventually to the muscles connected with them. We conclude from this that with equal illumination of both retinæ the symmetrical groups of muscles of both halves of the body will receive equal chemical stimuli and thus reach equal states of contraction, while, when the rate of reaction is unequal, the symmetrical muscles on one side of the body come into stronger action than those on the other side. The result of such an inequality of the action of symmetrical muscles of the two sides of the body is a change in the direction of movement on the part of the animal." And ('18, p. 83): "Motile plant organisms like *Volvox*, are driven to the source of light, owing to differences in the tension of the contractile organs on the shaded and illuminated sides and the same is true for animals like insects." Garrey says ('17, p. 609): "These experiments remove, in our opinion, the last doubt that the motions of animals to or from a source of light are due to an influence of the light on the tension of muscles of different sides of the body. . . . ." Minnich says ('19, p. 406): "I have not been able to observe any constant and pronounced difference in the muscular tension on the two sides of the body in the honey-bee. . . . I do believe that orientation is produced in this manner, however." Bohn ('09 a, p. 9; b, p. 4) and Patten ('19, pp. 453-457) make similar statements.

It is evident that according to all of these views the direction of locomotion in insects depends upon the relation between the effect of the activity of the locomotor appendages on opposite sides. Consequently, if these views are correct, the organism should turn continuously toward one side if the appendages on one side are thrown out of action, i.e., it should make circus movements. The results obtained in the following experiments have a direct bearing on this matter. It is, of course, self-evident that if the muscles in the locomotor appendages on opposite sides act precisely the same in an organism which is perfectly bilaterally symmetrical, it will take a straight course, and consequently that when it turns, these muscles do not act alike, but it does not follow from this that orientation is necessarily dependent upon a balanced effect in the action of these appendages, that the organism will turn continuously unless the muscles symmetrically situated in the appendages on opposite sides act equally, as is demanded by the views presented above.

Observations on the reactions to light in insects with some of the legs removed were made both on *Erax* and on *Eristalis*. Those made on the latter were, however, very much more thorough and extensive than those made on the former.

*Erax*. In some specimens of *Erax* the front leg and in others the front and the middle legs on one side were removed close to the body with a pair of scissors. These specimens were then, immediately or after one to twenty-four hours, exposed to various conditions of illumination. Without going into detail it may be said that the specimens with only one leg removed oriented nearly as precisely as normal specimens, there being only a slight tendency to deflect toward the normal side. When illuminated laterally they turned directly toward the light either to the right or to the left. In turning thus the front leg is extended laterally in either direction, fastened to the substratum and then flexed. In this way the anterior end is pulled either to the right or to the left until it faces the light after which the front leg is extended more nearly directly forward than it is normally and the animal proceeds toward the light.

The specimens with two legs removed oriented, on the whole, much less precisely than those with only one removed. Their movements were not well coördinated. They could readily turn toward the normal side but in attempting to turn in the opposite direction they usually fell over. However, they often proceeded fairly directly toward the light for considerable distances. The lack of precision in orientation appeared to be due largely, if not entirely, to the difficulty they had in remaining erect.

In the process of orientation under such conditions there is, of course, no possibility of a balanced effect of locomotor appendages in accord with the De Candolle-Verworn theory or with Garrey's tonus hypothesis.

*Eristalis.* In *Eristalis* orientation was studied in specimens with one front leg removed, with both front legs removed and with the front and the middle legs on one side removed. In some cases one eye was covered in addition to the removal of legs. In nearly all cases the accuracy of orientation was first ascertained by exposure in the dark room on dead black sheets of paper in a well-defined horizontal beam of light produced by a Nernst glower; then the legs were removed, after which the accuracy of orientation was again ascertained, either immediately or after the lapse of thirty minutes to three hours. In some cases the insects were then placed in low illumination or darkness and tested on each of the succeeding days until they became inactive. The reactions in nondirective light were also studied in a few specimens. The paths were recorded by following the insects with a pencil in such a way as to trace their courses on the sheets of black paper on which they were exposed. The direction of the rays was also recorded on each sheet.

In specimens with only one leg removed orientation was found to be very nearly as precise as it is in normal specimens and if anything more precise than in those with both front legs removed. This is clearly shown in the tracings of a typical series of paths reproduced in figure 5. These paths were all made by the same individual, two before the removal of any of the

legs, two immediately after the removal of one front leg and two immediately after the removal of both front legs. The removal of one of the front legs appeared to have very little effect on locomotion, but the removal of both caused a great reduction in the rate. By referring to the figure it will be seen that the insect when it had only one front leg, went very nearly as directly toward the light as it did when it had both, and even more directly than it did when it had no front legs. This seems to indicate that orientation is not inseparably associated with balanced action of symmetrical locomotor appendages on opposite sides.

In the specimens with the front and the middle legs on one side removed there was, in the first tests made after the operation, a decided tendency to deflect toward the normal side, both in the horizontal beam of directive and in the vertical beam of nondirective light. There was, however, considerable variation in the degree of deflection. One of the seven specimens studied under these conditions deflected nearly  $90^\circ$  in the horizontal beam while another oriented nearly as accurately immediately after the operation as it did before. All appeared to deflect more in nondirective than in directive illumination. No definite correlation was observed between the degree of deflection and the time between the operation and the first test. In the tests on succeeding days, however, the tendency to deflect decreased definitely in all but two of the specimens and one of these oriented almost perfectly immediately after the operation, while the other was in poor condition and died the second day following the operation. In some cases in which there was a decrease in deflection, there was later an increase again. This was especially marked one or two days before death. It seems to be related to weakness in the organism.

A typical series of paths obtained is represented in figure 6. By referring to this figure it will be seen that the specimen used oriented accurately before the operation, that it deflected strongly toward the normal side shortly after the operation, and that the deflection decreased from day to day until it had practically disappeared on the third day after the operation.

This shows that there is in *Eristalis* with the front and the middle legs on one side removed, improvement in the accuracy of orientation, which appears to be dependent upon experience, indicating a process of learning similar to that observed in other insects after the elimination of one eye, by Rádl ('03), Carpenter ('08), Holmes ('05), Dolley ('16), and Minnich ('19). It shows

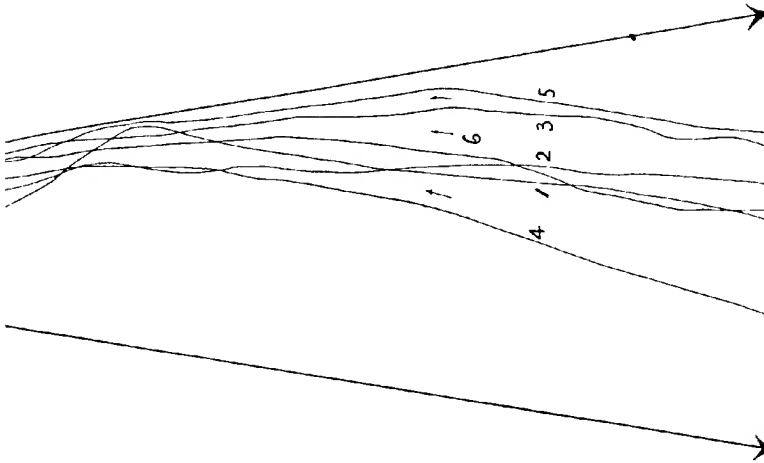


Fig. 5 Paths of *Eristalis* showing the effect on orientation of removing the front legs. 1, 2, paths of a normal individual; 3, 4, paths of the same individual with the right front leg removed; 5, 6, paths of the same individual with both front legs removed.  $x$ , point 20 cm. from 250 w. stereopticon lamp; small arrows, direction of movement of insect; large arrows, direction of rays and limits of beam of light.

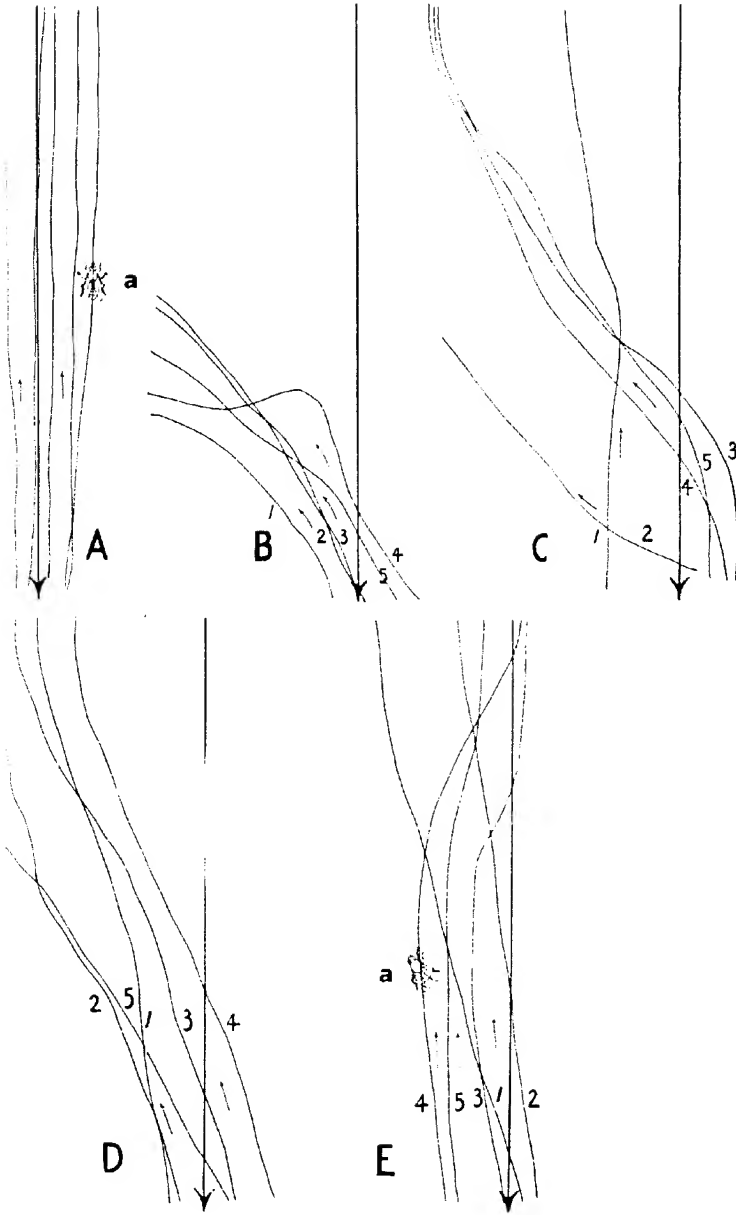
clearly that *Eristalis* with but one front and one middle leg can orient fairly accurately, and since it is chiefly these legs that are used in the process of orientation it is evident that orientation in these specimens is not the result of a balanced effect of symmetrically located locomotor appendages. Consequently orientation in *Eristalis* with the front and the middle legs on one side removed, cannot be accounted for by the De Candolle-Verworn theory or any other theory that demands balanced or antagonistic action in the locomotor appendages on opposite

sides. Rabaud ('21) reached similar conclusions in some observations on orientation to the vibrations of a tuning fork in the spider, *Argiope bruennicki*.

A detailed study of the movements of the feet in the photic responses of *Eristalis* with some of the legs removed leads to further important conclusions presented in the following pages.

After *Eristalis* with the front and the middle legs on one side removed, has assumed, in a horizontal beam of light, a course extending in a given direction which may be at various angles with the direction of the rays (fig. 6), it tends to retain this course. If the direction of the rays is changed by moving the source of light to the right or to the left it at once turns to the right or to the left until its course has approximately the same relation with the rays of light it originally had, i.e., it reorients, turning either toward the normal or the abnormal side. In turning toward the normal side the movements of the feet are very much like the movements in orientation in normal specimens. The front foot is strongly extended toward the light, and then attached to the substratum, after which the leg is flexed, thus pulling the animal toward the light. In turning toward the abnormal side, however, the movement of some of the feet seems to differ considerably from that observed in normal animals. In normal specimens the front leg opposite the side most highly illuminated seems to push the organism while in mutilated specimens it seems to pull it toward the light. In specimens with the front and the middle legs on one side removed it can be very clearly seen that, in turning toward the

Fig. 6 Paths of *Eristalis* showing effect on orientation of the loss of the front and the middle legs on one side. A, normal, 11/12, 3 p.m.; B-E, with right front and middle legs removed; B, immediately after the operation, 11/12, 3.15 p.m.; C, 11/13, 5 p.m.; D, 11/14, 4 p.m.; E, 11/15, 4 p.m.; 1-5, successive paths; a, axial position of oriented organism; large arrows, ray-direction at middle of beam of light; small arrows, direction of locomotion. All paths reduced by  $\frac{2}{3}$  linear dimensions. Note that after the legs were removed the insect deflected strongly toward the normal side, but that this deflection decreased from day to day until it practically disappeared. Note also that when the insect with the legs removed goes directly toward the light it goes sidewise and that the two eyes are not equally illuminated.



abnormal side, the front foot is extended forward, toward and beyond the median line (fig. 7), the precise direction depending upon the location of the light, and that it is then attached to the substratum after which the leg is flexed, pulling the animal toward the light. There is evidently, then, owing to the loss of two legs, a change in the action of the remaining legs such

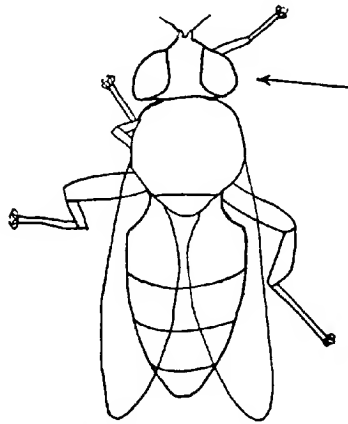


Fig. 7 Sketch indicating in *Eristalis* with right front and middle legs removed, the direction and extent of movement of the left front leg in turning to the right toward the light. Note that the front leg is well extended toward the right. In this position the foot is attached to the substratum after which the leg is flexed and the anterior end of the insect pulled to the right. Turning to the left is accomplished in the same way except that the front leg is extended to the left. The other legs assist in these movements. Arrow, direction of rays of light.

as to tend to maintain normal behavior in the organism as a whole. Changes observed in the function of the eyes lead to similar conclusions, as is indicated in the following paragraphs.

We have seen that immediately after the operation there is a tendency to deflect toward the normal side. This deflection is in part owing to a turning of the anterior end toward the normal side but it is mainly owing to a sidewise movement, the feet being extended somewhat laterally in place of directly forward, in such a way that when the insect directly faces the

light and the longitudinal axis is parallel with the rays there is still a deflection toward the normal side. Decrease in deflection is brought about in part by decrease in the lateral extension of the feet on the normal side (fig. 8) and in part by a change in the axial orientation so that when the insect is going directly toward the light the longitudinal axis makes an angle with the rays of light of about  $10^\circ$ . Consequently, when *Eristalis* with

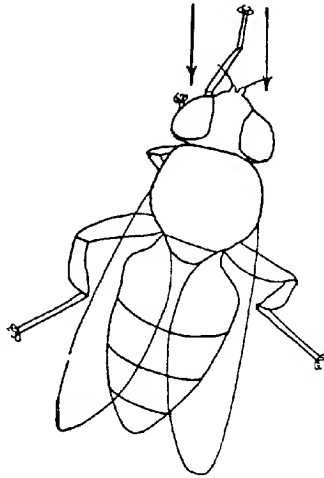


Fig. 8. Sketch showing *Eristalis*, with right front and middle legs removed, going toward the light nearly directly forward. Such specimens usually move somewhat sidewise in going toward the light. Arrows, direction of rays of light.

front and middle legs on one side missing is progressing directly toward a light it does not directly face the light and the two eyes are not equally illuminated (fig. 6, E), indicating that there has been a change in the relation between the location of the stimulus in the eyes and the direction of locomotion of such a nature as to tend to maintain normal behavior; in other words that this asymmetrical creature has learned that in order to go directly toward the light it is necessary to face toward a point somewhat to one side of the light so as to compensate for the lateral deflection owing to the loss of two legs.

It may be contended that the strong lateral progressive movement observed immediately after operation is due to stimuli produced by the injury resulting from the cutting of the legs and that the decrease in this lateral movement is owing to a gradual decrease in the magnitude of these stimuli as the wounds heal. There is, however, much evidence that militates against this contention.

1. In specimens with one leg removed there is very little if any deflection.

2. Specimens tested immediately after the removal of two legs do not appear to deflect more than those not tested until several hours later.

3. After marked decrease in the lateral deflection there was observed in certain specimens, a marked increase again. This was especially prominent some few days before death, and it is therefore probably, as previously stated, associated with a decrease in vitality.

4. For some time after the front and the middle legs on one side have been removed the hind leg on the same side appears to be paralyzed. At first it does not function at all. It is merely dragged along and in some instances it does not even prevent the fly from falling over. Gradually it assumes a more normal position and finally it functions apparently in perfect coördination with the other legs. The reactions of these legs, however, also become somewhat modified in the process of readjustment. Both the front foot and the middle foot are held somewhat nearer the median axis than they normally are, so as to prevent falling when the hind leg on the opposite side is raised in walking (fig. 8). Thus we find readjustments in the position of the legs in the fly somewhat similar to those found in a dog when it walks on three legs.

Now, all of these facts seem to indicate that decrease in deflection in specimens with the front and the middle legs on one side removed is, to say the least, not due entirely to a decrease in the stimulation of injury owing to healing of the wound, and that it is at least in part due to readjustment in the coördination of the action of the legs.

If the conclusions thus tentatively put prove to be correct, they will be of considerable interest for they will show that there is in *Eristalis* an innate tendency to proceed toward the light; a tendency of such a nature that if the light cannot be reached by the actions normally employed, others are used. Normally, *Eristalis* turns until the two eyes are equally illuminated and then proceeds directly toward the light. After the front and the middle legs on one side are removed it again tends to face the light directly, but owing to the absence of some of the legs it deflects and does not proceed toward the light. Later it tends to face toward a point some distance from the light. The two eyes are now unequally illuminated, but it proceeds directly toward the light. If it cannot go to the light in one way, it apparently employs another.

The results obtained in the observations on the reactions of *Eristalis* with one eye covered, as previously stated, seem to indicate that impulses originating in either eye may pass to and control the movements of the legs on both sides. The evidence we are about to present strongly supports this conclusion. This evidence was obtained in specimens of *Eristalis* with the front and the middle legs on one side removed and either eye covered. It can be most readily presented in connection with figures containing reproductions of typical series of paths made in a horizontal beam of light produced by a Nernst glower properly screened.

The paths reproduced in figure 9 were made by a specimen with the left front and middle legs removed and the right eye covered; those in figure 10 by one with the same legs removed but with the left eye covered.

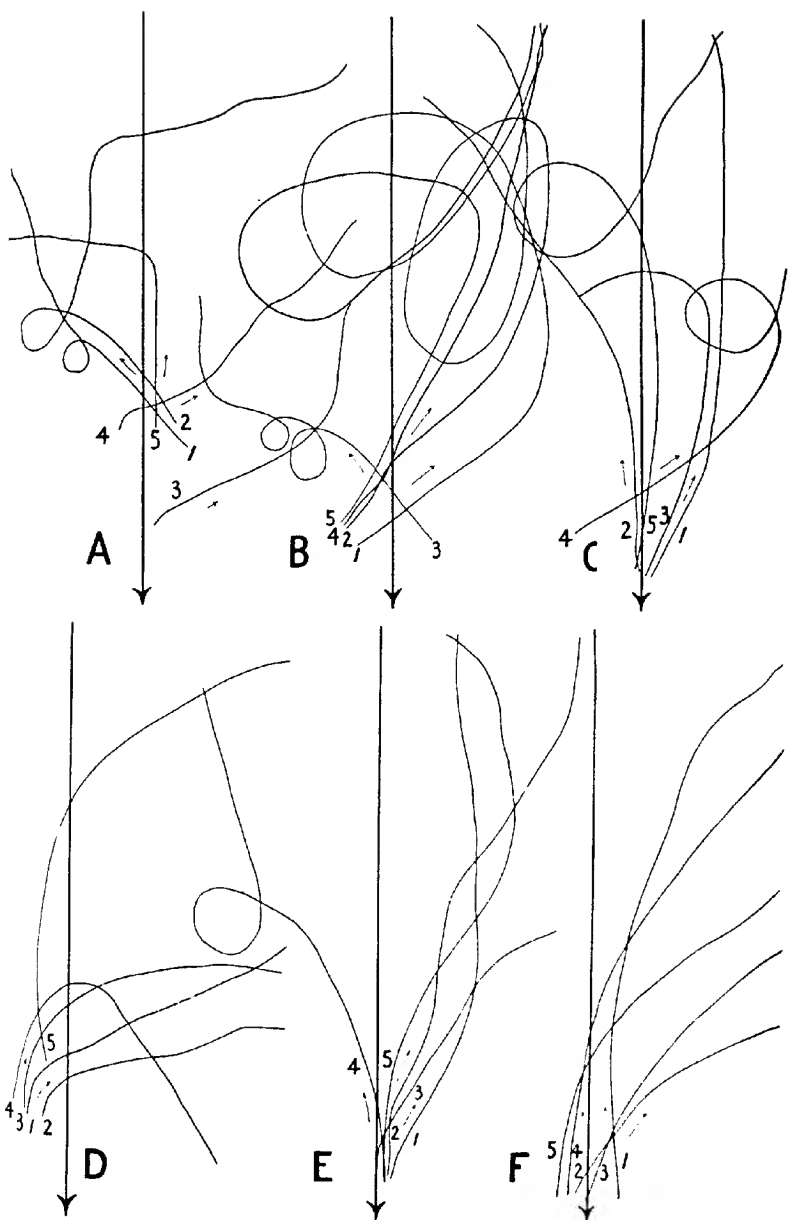
At the close of these experiments after the specimens had died the covered eyes of both specimens were carefully examined under a binocular. No openings were found; all of the facets were entirely covered. The outer surface of the eyes including the asphalt covering was then removed and examined under the compound microscope. There was no indication that any light passed through, even with the strongest illumination at hand. It is consequently certain that the sensitive tissues in

the covered eyes of the two specimens used in these experiments received extremely little if any light.

The first group of paths (A) reproduced in figure 9 were made November 5th shortly after the legs had been removed and the eye had been covered. The other groups (B-F) reproduced in this figure were made on November 6, 7, 10, 11 and 12, respectively. By referring to these paths it will be seen that the insect with the right eye covered and the left legs removed proceeded toward the light in all trials, but that there was, especially at first, a strong tendency to deflect both to the right and to the left. In paths 1 and 5 (fig. 9, A) it deflected strongly to the left, in paths 3 and 4 strongly to the right and in path 2 strongly to the left in the first part and equally strongly to the right in the second part. By comparing the paths on the various days it is at once evident that there was a definite decrease in the tendency to deflect. This is particularly marked in the paths made on November 11 (fig. 9, E). These paths show that in some of the tests made on this day the insect proceeded fairly directly toward the light deflecting but little either to the right or to the left. The deflections to the right which may be observed in all of the paths produced the following day, November 12th, was doubtless due to the unfavorable physical condition of the specimen, for it died several hours after these tests were made.

The first group of paths made by the specimen with the left eye covered and the left legs removed (fig. 10, A) was obtained November 11th, two days after the eye had been covered and two days after the front leg had been removed, but immediately after the middle leg had been removed. The following groups of paths were made on successive days November 12, 13, 14, 15, 16, respectively. These paths show that there was during the

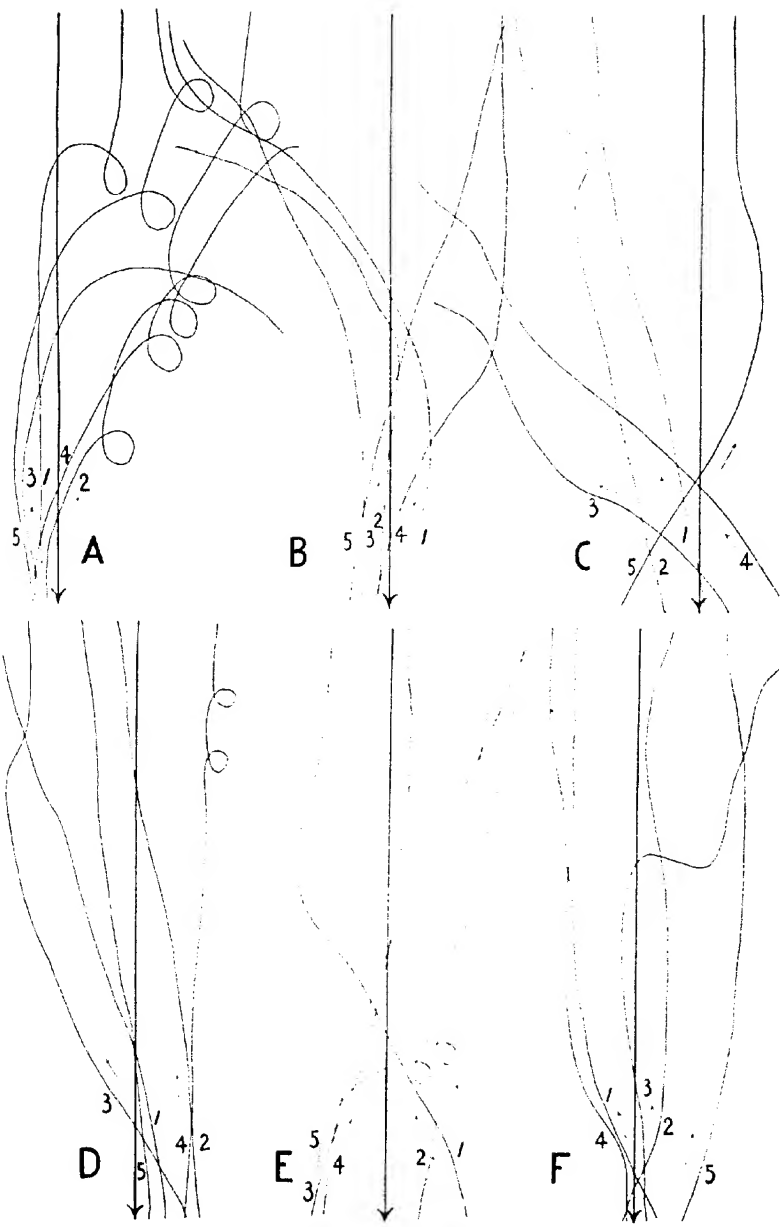
Fig. 9 Paths of *Eristalis* showing effect on orientation of the loss of front and middle legs on one side and the eye on the opposite side. A, 11/5, 3 p.m., immediately after removing left front and middle legs and covering right eye; B, 11/6, 3 p.m.; C, 11/7, 4 p.m.; D, 11/10, 4 p.m.; E, 11/11, 10 a.m.; F, 11/12, 3 p.m.; 1-5, successive paths; small arrows, direction of locomotion; large arrows ray-direction at middle of beam. Paths reduced by  $\frac{1}{3}$  linear dimension.



first set of tests (fig. 10, A) a strong tendency to deflect toward the side containing the functional eye and the normal number of legs, that this tendency decreased rapidly so that on the following day it had practically disappeared, the deflections to the left having been fully as great as to the right (fig. 10, B), and that there was marked improvement in the accuracy of orientation, the movement toward the light in the last group of tests (fig. 10, F) having been surprisingly direct.

This specimen lived until November 22nd, but no more tests were made. In both specimens, but especially in the one with the right eye covered, there was at first considerable difficulty in locomotion evidently owing to improper coördination. This was particularly noticeable in the hind leg on the left side, the side from which the front and the middle legs had been removed. Improvement in the accuracy of orientation was in part due to improvement in coördination, but it was also affected by an apparent change in the relative sensitiveness of different parts of the eye. At first circus movements toward the functional eye were prevalent in both specimens, but more so in the one with the functional eye and legs on the same side than in the one with these on opposite sides. Later the circus movements disappeared entirely. This was probably owing to a decrease in the sensitiveness of the lateral and the posterior portion of the eye in relation to that of the anterior portion. This is supported not only by the fact that the tendency to deflect toward the functional eye decreased but also by the fact that, while at first light directed toward the anterior surface of the right eye from the left did not induce turning toward the left, i.e., toward the side with the covered eye, later under precisely the same conditions it did induce turning in this direction, indicating clearly that there was a change in the relative sensitivity of the different portions of the eye.

Fig. 10 Paths of *Eristalis* showing effect on orientation of the loss of front and middle legs and the eye on one side. A, 11/11, 12 m. immediately after removing middle left leg and two days after removing front left leg and covering left eye; B, 11/12, 2.45 p.m.; C, 11/13, 5 p.m.; D, 11/14, 4.30 p.m.; E, 11/15, 3 p.m.; F, 11/16, 2.10 p.m.; 1-5, successive paths; small arrows, direction of locomotion; large arrows, ray-direction at middle of beam. Paths reduced by  $\frac{1}{3}$  linear dimension.



Both specimens, the one with the left eye covered as well as the one with the right eye covered, in moving toward the light invariably oriented so that the longitudinal axis made an angle of approximately  $30^\circ$  with the direction of the rays, the normal side facing the light. They were in fact pulled sidewise toward the light by alternate lateral extension and flexure of the legs on the normal side. The hind leg on the opposite side did not appear to take any part in the process. In some instances it was indeed very evident that it was being dragged along without any indication whatever of normal stepping movements.

If the light was moved either to the right or to the left after the insects had assumed a definite axial position, they at once turned either to the right or to the left until they again had the same axial position they had had before the direction of the rays was changed. This proves conclusively that the movements of the legs may be controlled by impulses from either eye. If, e.g., the light is directed toward the lateral surface of the right eye, the front right leg is extended laterally to the right and the animal is pulled toward the light; if it is directed toward the lateral surface of the left eye, this leg is extended forward and toward the left beyond the median plane and the animal is again pulled toward the light (fig. 7). In these reactions the remaining legs on the same side respond in such a way as to facilitate the result of the action of the front leg. Thus it is pulled toward the light either to the right or to the left by the same legs depending upon whether the right or the left eye is illuminated. Illumination of a given region of the retina in one eye induces a given set of reactions in the legs on one side, illumination of the same region in the other eye induces reactions in the same legs but of a quite different sort. These are clearly actual responses, responses similar to those observed in the scratch-reflex in dogs or cats induced by local stimulation of the skin. They depend in character upon the location of the stimulus in the former just as they do in the latter and they can no more be accounted for on the basis of tonus-effects in accord with Garrey's postulate ('18, p. 110) in the one than in the other.

Thus it is evident that the movements of the legs on both sides may be controlled by impulses originating in either eye, and that in normal specimens stimulation of a given region of the retina in one eye induces simultaneously reactions in the legs on both sides, of such a nature, however, that the effect of those induced in the legs on one side is facilitated by the effect of those simultaneously induced in the legs of the other side. We have demonstrated previously that the nature of these reactions depends upon the location of the stimulus in the eye, and that the illumination of different regions of the eye induces different sets of reactions in the legs: so that, within certain limits, stimulation of any given region of the retina is followed by certain specific reactions, differential responses to localized stimulation. We have also demonstrated that reactions induced by stimulation of any given region of the retina in one eye are modified by simultaneous stimulation of another region of the retina in the same eye. What, now, occurs when the same region of the retina is simultaneously stimulated in both eyes? What occurs when these regions are not the same in location? What occurs when the stimuli in the two eyes differ in magnitude, and what occurs when they are equal?

The experiments on orientation in light from two sources, considered in the following pages have a bearing on these questions. They are of great importance for they have a direct bearing on the process of orientation in normal specimens.

#### ORIENTATION IN LIGHT FROM TWO SOURCES

Observations on the reactions in light from two sources were confined almost entirely to *Eristalis*. But in this form the reactions were studied in specimens both on foot and on the wing. Moreover, in some of the specimens studied on foot one eye was covered.

In these observations it was found that *Eristalis* on foot orients nearly as precisely in light from two sources as it does in light from a single source. If specimens are exposed at the intersection of two beams of light which cross at right angles they go toward a point between the two beams. If the light

in these beams is equal in intensity this point is located approximately half way between the beams but if it is not equal the point is located nearer the more intense beam and the greater the difference the nearer this beam it is located. This, the results obtained clearly demonstrate, but the precise location of the point in question in relation to the relative intensity of the two beams has not as yet been ascertained.

These results are in harmony with those obtained by various investigators on numerous other organisms. They probably hold for all invertebrates and for some vertebrates especially those with poorly developed eyes. (Mast, '07, '11; Patten, '14; Dolley, '16; Buder, '17, et al.). Loeb's contention ('05, pp. 2, 82, 268) that if the light received from the two sources is unequal the animals go directly toward the more intense, while if it is equal they go toward a point half-way between, receives no support. Some of the higher animals, e.g., the toad (Mast, '11) do go directly toward the more intense source if the two are unequal,<sup>6</sup> but they do not go toward a point half-way between if they are equal. Loeb's methods were very crude. He made his observations in light received from two windows and did not measure the intensity. This doubtless accounts for his error.<sup>7</sup>

Buder ('17) in some very thorough work on *Euglena* and *Chlamydomonas* obtained results which seem to prove conclusively that the law of parallelogram of forces holds for these forms in so far as it refers to the relation between direction of

<sup>6</sup> Dolley maintains that this also holds for some insects, especially the bumblebee under certain conditions (personal communication). Buddenbrock ('17) obtained results which indicate that it also holds for certain caterpillars.

<sup>7</sup> Loeb says ('18, p. 75): "The writer observed that if heliotropic animals are exposed to two equidistant lights of equal intensity they move in a line perpendicular to the line connecting the two lights," and in this connection he refers to previous works published in 1890 and 1905. In these references ('90, p. 74, and '05, p. 61) he says: "When the diffuse daylight which struck the larvae came from two windows the planes of which were at an angle of 90° with each other, the paths taken by the larvae lay diagonally between the two planes." In light received from two windows as described above were the animals actually "exposed to two equidistant light: of equal intensity," as the author maintains? If not, what basis is there for his contention?

movement and the relative illumination received from two or more sources. Loeb maintains that this holds also for *Littorina*, but he presents no evidence in support of his contention. He says ('13, p. 473): "Eine *Littorina*, die zwischen zwei anziehende dunkle Schirme . . . gesetzt wird, wird von beiden angezogen und bewegt sich infolgedessen zwischen beiden, dem Parallelogramm der Kräfte entsprechend." The results obtained by the present writer on *Volvox* ('07) and on blowfly larvae ('11) indicate that it does not hold for these forms. These observations should be extended. They have a direct bearing on the problem of orientation as will appear in the following pages.

Loeb in various statements regarding orientation maintains that whenever an animal is oriented in light the photo-receptors on opposite sides receive the same amount of light. He says, e.g., ('13, p. 473), referring to orientation of *Littorina* in light from two sources: "Das Tier stellt sich so ein, dass beide Augen gleichmässig erleuchtet sind." ('18, p. 47.) "In the case of unequal illumination of the two eyes the tension of the symmetrical muscles in an animal becomes unequal. In this condition the equal impulses of locomotion will result in an unequal contraction of the muscles on both sides of the animal. As a consequence the animal will turn automatically until its plane of symmetry is in the direction of the rays of light. As soon as this happens the illumination of both eyes and the tension of symmetrical muscles become equal again and the animal will now move in a straight line--either to or from the source of light" (see also '18, pp. 70, 75, 79, 93). Patten ('14) supports this contention, at least in so far as it refers to the blowfly larva. Does it hold for insects?

When an insect is oriented and proceeds toward light from a single source or when it proceeds toward a point half way between two sources of light of equal intensity and at equal distances the two eyes receive the same amount of light and they are equally illuminated (fig. 11), but when it is oriented in light from two sources of unequal intensity, and proceeds toward a point which is nearer one than the other this is not true, the

eyes are not equally illuminated and they do not receive the same amount of light; this is demonstrated in the following paragraphs.

An insect which is oriented in light from two sources of equal size and distance but of different intensities faces a point nearer one than the other as previously stated. If the relation between the intensity of the light in the beams produced by the two sources is such that the angle between the path of the oriented insect and the stronger beam is more than a certain number of degrees, approximately 15 for *Eristalis*, the light in either beam, owing to the direction of the ommatidia reaches the retina of only one eye. That is, the retina of the right eye receives light only from the source to the right and that of the left eye only from the source to the left. The light received is in the form of images of the two sources of light, one on the retina of either eye. These two images are equal in size but they differ in brightness and in location (fig. 12). The brighter image is farther forward than the other and the greater the difference in brightness the greater the difference in location. If the angle mentioned is less than the stated amount both eyes receive light directly from the stronger beam, that is, one eye receives light directly from two sources and has two images on the retina, while the other eye receives light from only one and consequently has only one image (fig. 13). These images are all approximately the same in size but they differ in brightness. It is, consequently, evident that when the insect is oriented under such conditions the two eyes are not equally illuminated and they do not receive the same amount of light. Moreover, Parker ('03) and Cole ('07) clearly demonstrated that the stimulating efficiency of light depends upon the size of the image on the retina, a given amount of light spread over a large area having a greater stimulating effect than the same amount spread over a small area. This may be due either to the fact that more rhabdomes are illuminated in the one case than in the other or to the fact that the illuminated area extends farther back in the one than in the other. These facts show conclusively that orientation is not necessarily dependent upon

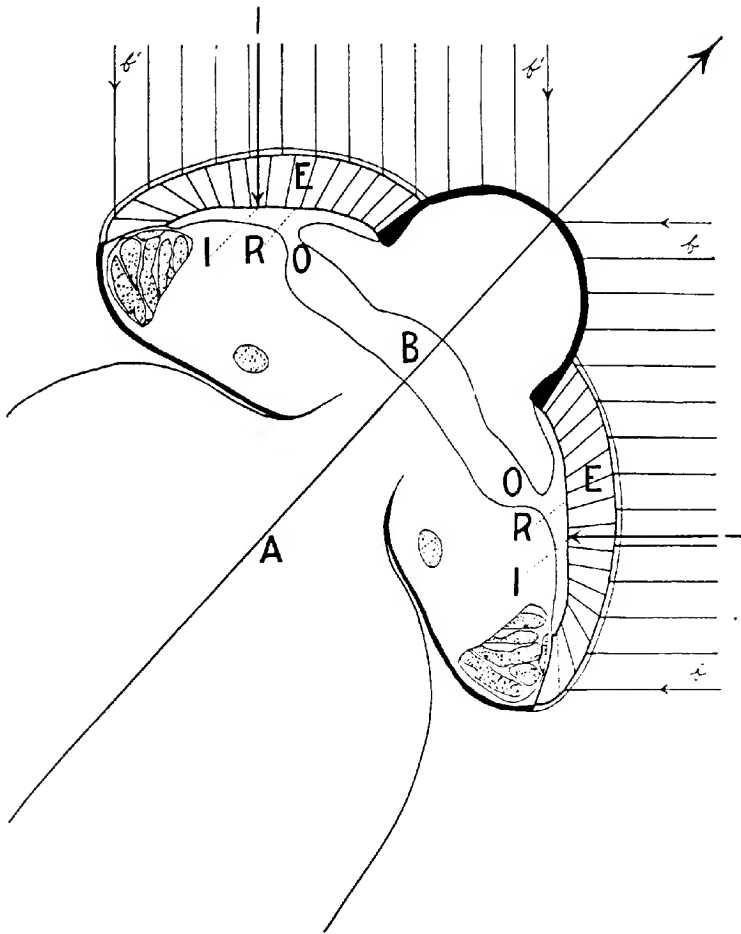


Fig. 11. Camera outline of a horizontal section of the head of *Eristalis* showing location of stimuli in specimens oriented in light from two sources at equal distances and of equal size and intensity. *b-b* and *b'-b'*, horizontal beams of light from two lamps so far removed that the rays are practically parallel; *A*, longitudinal axis of insect; *E*, eye with lines showing accurately the direction of the longitudinal axis of the ommatidia in different regions; *B*, brain; *O*, optic nerve; *R*, retina; *I*, image of lamp on the retina. Note that when an insect is oriented in light from two sources of equal size and intensity and at equal distances, the two eyes are equally illuminated, there being one image in either eye, both located in the same relative position and equal in size and luminous intensity.

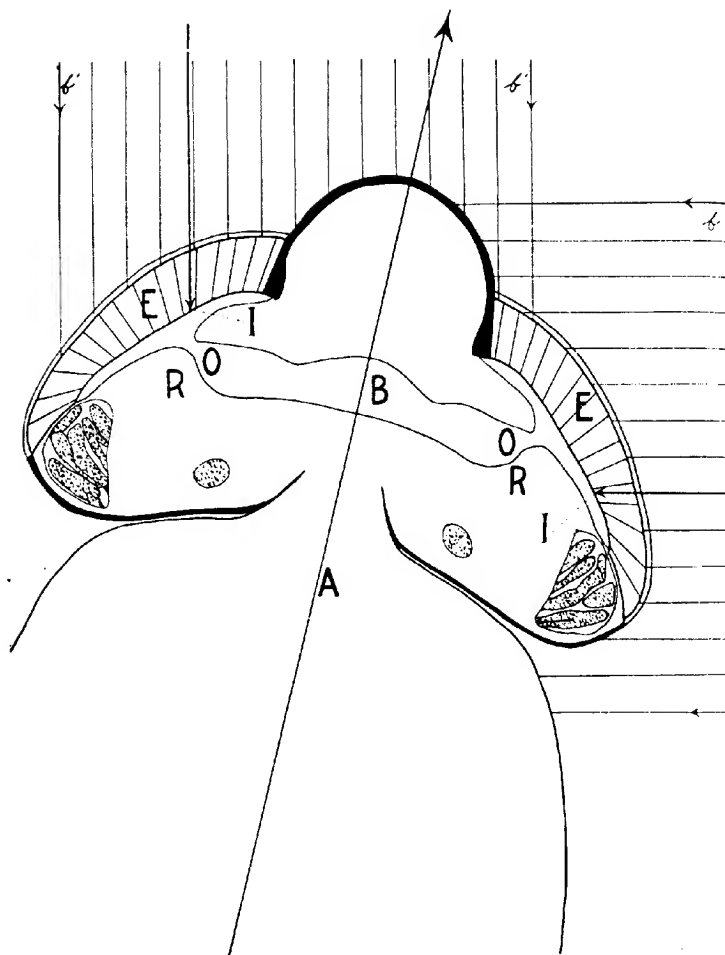


Fig. 12 Camera outline of a horizontal section of the head of *Eristalis* showing location of stimuli in specimens oriented in light from two sources at equal distances and of equal size but of different intensities.  $b$   $b$  and  $b'-b'$ , horizontal beams of light from two lamps so far removed that the rays are practically parallel;  $A$ , longitudinal axis of insect;  $E$ , eye with lines showing accurately the direction of the longitudinal axis of the ommatidia in different regions;  $B$ , brain;  $O$ , optic nerve;  $R$ , retina;  $I$ , image of lamp on the retina. Note that the image in the left eye (the stronger image) is located much farther forward than the image in the right eye (the weaker image); and that when an insect is oriented in light from two sources of unequal intensity the two eyes are not equally illuminated.

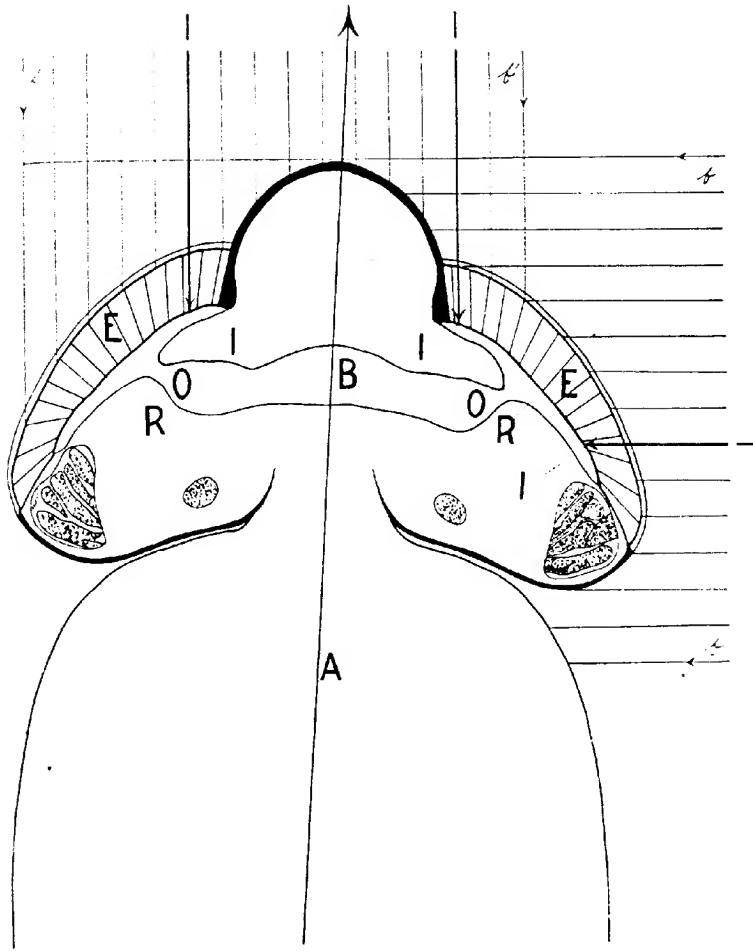


Fig. 13 Camera outline of a horizontal section of the head of *Eristalis* showing location of stimuli in specimens oriented in light from two sources at equal distances and of equal size but of greatly different intensities. *b-b*, and *b'-b'*, horizontal beams of light from two lamps so far removed that the rays are practically parallel; *A*, longitudinal axis of insect; *E*, eye with lines showing accurately the direction of the longitudinal axis of the ommatidia in different regions; *B*, brain; *O*, optic nerve; *R*, retina; *I*, image of lamp on the retina. Note that when *Eristalis* is oriented in light from two sources which differ greatly in intensity the two eyes are not equally illuminated, there being a strong image in one eye and a weak and a strong one in the other, all differing in relative location.

equal illumination of the two eyes as is maintained by Loeb, Patten,<sup>8</sup> and others, and they indicate that when insects are oriented under natural conditions, the two eyes are rarely if ever equally illuminated.

The results presented above support the conclusion, previously reached, that a given amount of light received by a given area near the posterior border of the retina has a greater effect in inducing turning reactions than the same amount of light received by an equal area of the retina near the anterior border; that there is, as one proceeds anteriorly, a gradual reduction in the sensitivity of the retina as measured by the effect of a given amount of light on turning. They show, moreover, that the turning effect of a given amount of light received by a given area of the retina in either eye is neutralized if the same amount of light is received by the same area in the other eye, or if less light is received by an area farther back in this eye. In other words, they show that to neutralize the turning effect of a given photic stimulus in one eye it requires in the other eye less and less energy as the area stimulated in this eye approaches the posterior border of the retina, provided the areas illuminated in the two retinas are the same in size and form.

In *Eristalis* on the wing the results obtained were very much less definite than those obtained in *Eristalis* on foot. They show, however, that when this insect is exposed to light from two sources it tends to fly toward a point between the two sources no matter whether these sources are on a horizontal or on a vertical line.

<sup>8</sup> Patten ('14, pp. 262-267), in a valuable paper on blowfly larvae, assumed that when these organisms are oriented, the photo-receptors on opposite sides always receive the same amount of light. On the basis of this assumption he made extensive calculations and concluded that the photo-receptors, which are entirely unknown as to location, form and structure, must be inclined toward each other at an angle, that they can not consist of "fixed eyes so placed that the tangents to the eyes at the optical axes are parallel." Loeb ('18, p. 79) accepts this conclusion. The facts presented in this paper indicate, however, that the assumption on which the conclusion rests is not valid for flies, and those presented by Taliaferro ('20) indicate that it does not hold for flatworms; it consequently does not seem likely that it will hold for blowfly larvae.

The observations on specimens on the wing were all made in the dark room. Two 40-watt mazda lamps were mounted in opaque boxes, screened and arranged in such a way as to produce rather large beams of light which crossed each other at an angle of about  $30^\circ$ . The lamps were 125 cm. apart. The flies were then liberated one at a time at a point 400 cm. from either lamp.

When the lamps were on a horizontal line practically all of the flies flew toward a point between the lamps. The location of this point varied greatly, however, with different individuals and with the same individual in different tests. When the lamps were on a vertical line the results obtained were even more variable than when they were on a horizontal line. In some series of tests practically all of the specimens flew toward various points below both lamps. In a few series a majority flew towards points above both lamps. In others, however, a great majority flew toward points between the lamps. The effect of gravitation is undoubtedly involved in this inconsistency. It was noted that when the insects were not very active, as e.g., in low temperature, they invariably tended to go down as they flew toward the light, and that when they were most active they tended to fly toward points between the lamps. Why they sometimes persistently flew upwards I am unable to say. That there is, however, under the influence of light alone, a tendency to fly toward a point between two sources of light is clearly indicated by the following results.

On October 14th, 14 flies were tested. Of these 9 flew toward points between the two lamps, 4 toward the upper lamp or points above, and 1 toward a point below the lower lamp.

On October 17th, 19 flies were tested. Of these, 2 flew toward points between the lamps, 17 toward the lower lamp or points below and none toward the upper lamp or points above. Fourteen of these specimens, the rest having been lost, were now tested with the lamps on a horizontal line. One flew toward a point between the lamps, 13 toward points below the lamps and none toward points above.

On October 21st, 31 flies were tested. Of these, 16 flew toward points between the lamps, 10 toward the lower lamp or points

below and 5 toward the upper lamp or points above. Thirty of these flies, one having been lost, were then tested with the lamps on a horizontal line. Of these 17 went toward a point between the lamps, 8 toward points below and 5 toward points above the lamps.

These results indicate that when *Eristalis* on the wing receives light of equal intensity from two sources of the same size it tends to go toward a point halfway between them no matter whether the sources of light are on a horizontal or on a vertical line.

When an insect at equal distances from two sources of light on a vertical line proceeds toward a point midway between the two sources, that is, when it is oriented under such conditions, the two eyes are equally illuminated and receive the same amount of light, but there are two images on the retina of each eye, one above and the other below the center of the anterior surface. If either of these images is eliminated in both eyes, e.g., by turning off one of the lamps, the insect turns upward or downward until it faces directly the remaining lamp (fig. 3), i.e., until the two remaining images assume central positions in reference to the anterior surfaces of the eyes. This indicates that when an insect is oriented in light from two sources on a vertical line, the turning effect of either image in either eye is eliminated by the effect of the other image in the same eye. This conclusion is supported by the results obtained in the following observations.

If specimens of *Eristalis* with one eye blinded are exposed on a horizontal surface in a well-defined beam of light they turn, as previously stated, toward the functional eye until the longitudinal axis makes a given angle with the direction of the rays, which varies greatly with different individuals and with the same individual at different times, then they proceed in a fairly straight course until they approach the edge of the beam, where they usually turn and go nearly directly toward the light, remaining continuously in the beam of light but very near the edge. If the direction of the rays is changed after they have become oriented in the beam, they immediately change their

direction of locomotion until their course has the same angle with the rays of light that it formerly had. In this process, they may turn either toward the blind side or toward the normal side.

Dolley ('16) repeatedly observed similar responses in the mourning-cloak butterfly. He found that when specimens with one eye covered are exposed in a well-defined beam of light, they tend to cross the beam at an angle, that they tend to turn toward the light as they approach the edge of the beam and that the angle of deflection, which varies greatly, is independent of the intensity. That is, that the course taken makes approximately the same angle with the rays regardless of the distance from the source of light.

It is well known that illumination of the lateral and posterior portions of the retina induces turning toward the eye stimulated; and there is considerable evidence indicating that illumination of the antero-median edge induces turning in the opposite direction, while illumination of the central portion of the anterior part does not induce turning in either direction.

When the insect in the beam faces the light directly there is an image of the source of light on the retina located in the central portion of the anterior part of the eye (fig. 13). This, owing to its location tends to induce locomotion directly forward, but the retina also receives a certain amount of light reflected from the background and this is so distributed that the entire lower portion of it is illuminated. The illumination of the lateral and posterior portion of the retina, faint as it is in comparison with that in the image of the source of light located at the anterior end, causes the insect to turn toward the functional eye. As the insect turns the image of the source of light travels toward the antero-median edge of the retina, where it disappears, when the insect has turned far enough so that the longitudinal axis makes an angle of about  $15^\circ$  with the rays of light (fig. 13). After this the retina receives light only from the background but the antero-median edge receives much more than the posterior portion. The former tends to induce turning toward the blind side the latter toward the normal side. As

the insect turns the illumination of the retina decreases, but it decreases more rapidly on the posterior portion than it does on the anterior. The tendency to turn in the two directions consequently becomes neutralized, the insect becomes oriented and proceeds diagonally across the beam in a fairly straight course. However, as it approaches the edge of the beam the reflected light received by the retina from the background becomes gradually weaker, but that received by the posterior portion decreases more rapidly than that received by the anterior portion. The insect consequently turns toward the blind side, and proceeds toward the source of light in close proximity with the edge of the beam where the lateral and posterior portions of the retina receive but very little light reflected from the background.

These facts and considerations indicate that the turning effect of the illumination of the antero-median edge of the retina is neutralized by simultaneous illumination of the postero-lateral portion and that this neutralization requires relatively less and less light as the posterior end of the eye is approached.

The evidence at hand, then, seems to indicate that in insects the turning effect of local illumination in the retina of one eye is inhibited by equal illumination of the same region in that of the other eye or by weaker illumination of a region relatively farther back in either eye. But how under these conditions of illumination is turning inhibited? It is evident that it could be inhibited by total inhibition of the effect of the illumination on the legs, or by an equal effect on the legs on opposite sides, such that they tend to move at the same rate or pull or push in opposite directions equally.

Loeb, Bohn and others, as previously stated, imply that it is prevented by a balance in the rate of the action of the legs on opposite sides. They hold that when an insect is not oriented and the two eyes do not receive the same amount of light, the rate of photochemical changes in them differs, and that owing to this difference, the activity of the legs on opposite sides differs in such a way that the feet on one side move faster than those on the other, causing the animal to turn until the two

eyes are equally illuminated and the photochemical changes are the same, resulting in an equal rate of movement on opposite sides and consequently no further turning. They maintain that the orienting stimulus operates after orientation as well as during the process of orientation; that the rate of movement bears some specific relation to the amount of light received by the eyes; that the rate of movement of the feet on one side is controlled by impulses received from one eye and that of those on the opposite side by impulses received from the other eye. If this actually obtains the rate of locomotion in oriented specimens should be specifically related to the intensity of the illumination. Dolley ('17) in a very thorough study on *Vanessa* found, as previously stated, that the rate of locomotion over a wide range is practically independent of variations in luminous intensity. Holmes ('03) obtained similar results in observations on *Volvox*. Herms ('11) found, however, that fly larvae travel more rapidly in high than in low illumination, as did also the writer ('11), but the difference in the rate of locomotion found is so small in comparison with the difference in the intensity of the light that it can have no practical bearing on the problem of orientation. Loeb ('90, p. 59) maintains that aphids walk twice as fast in direct sunlight as in diffuse sunlight, and he concludes that there is a very definite relation between illumination and rate of locomotion. Moore and Cole ('21) maintain that in *Popillia japonica* the relation between the rate of locomotion and luminous intensity follows the Weber-Fechner law. There is, however, much doubt concerning the validity of these contentions, for Loeb in his observations did not exclude the effect of difference in temperature and Moore and Cole did not exclude the time required for the insects to orient and to get under way. It is consequently clear that the evidence at hand indicates that the neutralization of the turning effect of illumination in insects is not due to equalization of the rate of movement on opposite sides.

Garrey ('18) contends, as previously stated, that orientation in insects is dependent upon the effect of light on the tonus of the muscles in the legs, that when the two eyes are unequally

illuminated the legs on one side are more extended than those on the other and that this results in turning until the eyes are equally illuminated and the effect on tonus is equal on both sides. He consequently holds that the light received by the eyes operates in producing tonus after orientation precisely the same as during the process of orientation. Now, if all of these contentions obtain we should expect in oriented insects a specific relation between the degree of extension in the legs on both sides and the luminous intensity to which the eyes are subjected. In strong light the legs on both sides should be well extended, in weak light well flexed or vice versa depending upon whether the reactions of the legs are controlled by impulses received from the eye on the same or on the opposite side. If the neutralization in question is accomplished by equality in movement in opposite direction on the two sides the degree of the extension of the legs should also be related to the amount of light received by the eyes. In strong light the legs on both sides should be markedly more extended than in weak light.

Detailed observations on *Eristalis* were made in reference to this matter, as previously stated. In these observations I exposed specimens in light varying in intensity from nearly darkness to more than 50,000 m.c. In some cases illumination was rapidly changed, in others slowly. In some cases the specimens were in motion when the changes were made, in others they were at rest. Similar observations were also made on *Erax*. In none of these observations was there any definite change in the degree of extension of the legs correlated with changes in the amount of light received by the eyes. Consequently, if there is any relation between illumination and the degree of extension of the legs in oriented specimens of *Eristalis* or *Erax* it is certainly very slight. It may, therefore, be concluded that in these forms, when they are oriented, the turning effect of the light is neutralized by inhibition, and not by equal tonus or by equal action in the legs on opposite sides; that the orienting stimulus ceases after orientation has been attained; that the effect of photochemical or other changes in one eye is in some way inhibited by similar changes in the other eye.

These results also indicate that the tilting in one-eyed organisms observed by Rádl ('03), Holmes ('05), Mast ('11), Garrey ('18), and others is prevented, under conditions of equal illumination of the two eyes in normal animals, by inhibition of the reactions which produce it and not by an equality in the reactions on opposite sides as Garrey ('18) seems to hold.

How this inhibition is accomplished is not known, but the central nervous system is certainly involved. It may be that it is accomplished by interference in the impulses originating in the two eyes in accord with Verworn's theory of inhibition ('13, pp. 206-234). Results recently obtained by Dolley ('20) lend some support to this view. This matter will be referred to in some detail in the following section on the nature of photic stimulation.

The results obtained in the observations on the reactions in light from two sources demonstrate, then, that when insects are oriented under such conditions, the two eyes are not equally illuminated unless the two sources of light are equal in size and intensity and at equal distances from the eyes. They indicate that the two eyes are rarely if ever equally illuminated in insects oriented under natural conditions. And they show that when an insect is oriented the light has no immediate observable effect on the legs, that the stimulating effect of the illumination of one eye is inhibited in the central nervous system by the simultaneous illumination of the other eye.

But if the insect is not stimulated after it is oriented and thereby continuously held upon its course, as the De Candolle-Verworn theory demands, how is it that it can proceed so directly toward a source of light? It is well known that insects in the absence of photic stimulation proceed for considerable distances in fairly straight courses, consequently, if the orienting stimulus ceases after they are oriented, one would expect them to continue for some distance directly toward the light. But if they do turn the location of the region illuminated in the eyes immediately changes and this results in stimulation and reorientation. By referring to figure 11 it can readily be seen how the location of the luminous images in

the eyes change with changes in the axial position of the insects. If the insect, as represented in this figure, turns to the right, e.g., the image in the right eye moves forward and that in the left eye moves backward. Thus even a minute deviation to the right or to the left from the position of orientation results in a considerable change in the relative position of the images in the two eyes. The amount of change required to induce orienting responses is not known but it is probably very small, so that only very slight deviations from the oriented position would be expected.

#### THE NATURE OF THE STIMULUS<sup>9</sup>

It has been well known for many years that in certain reactions the stimulus depends upon the time-rate of changes in the intensity of the stimulating agent, while in others it does not.<sup>10</sup> *Euglena*, e.g., responds under certain conditions, by suddenly turning toward one side if the light is rapidly decreased, but not if it is slowly decreased, no matter how great the decrease may be. Under other conditions it responds only if the light is rapidly increased. Engelmann ('82, p. 395) designated these reactions 'Schreckbewegung'. Similar reactions have been observed by various investigators in many other organisms (Mast, '11, p. 247). They are in fact found in practically all groups of animals from *Ameba* to man.

<sup>9</sup> The term stimulus is used here in a general sense including all changes both external and internal involved in the production of impulses and reactions. It thus includes processes which occur in the environment (the stimulating agent) and processes which occur in the receptors (the stimulus in the restricted sense).

<sup>10</sup> Loeb says ('18, p. 119): "When a galvanic current is sent through a motor nerve the muscle answers with a contraction only when the current is made or broken, but not while a constant current is flowing through the nerve. The older physiologists were not able to form a mental picture of what happened in this case, and they cut the knot by invoking a verbalism, namely by calling the making or breaking of a current a 'stimulus'. Thus Jennings and Mast took it for granted that phenomena of orientation by light could only be produced by rapid changes in the intensity of light and not by constant illumination, since they had the *a priori* conviction that only a rapid change in the intensity of a galvanic current or of light is a 'stimulus'."

These reactions are clearly of the 'trigger' or 'all or none' type. They consist either of a single well-coordinated response or of several such responses in succession, after which the reaction ceases until the light is again changed. It is quite generally assumed that they are dependent upon photochemical changes in the receptors, and it is altogether probable that the accumulation of a given amount of some specific photochemical substance is necessary to induce them, but it is clear that the ac-

I am at a loss to know how Loeb reached the conclusion stated in the preceding quotation regarding the ideas of "the older physiologists," Jennings and Mast, concerning a "stimulus." He must have overlooked two sections in Jennings' book entitled "Reaction to the Constant Current" ('06, pp. 83-89 and pp. 152 and 164) dealing exclusively with reactions dependent upon the continuous electric current, and numerous statements in my writings. For example, in my book ('11, p. 163) I say: "In the orientation of this organism it seems probable that light acts as a continuous directive stimulation" (p. 208). "There is direct evidence showing that the organism (*Planaria*) responds both to changes of intensity and to constant intensity," and (p. 223) "In these reactions light no doubt acts continuously as an orienting stimulus." Moreover, similar statements will be found on pages 111, 144, 162, 164, 173, 175, 206, 209, 210, 223, 233, 234, 245, 252, 253 and 257. Furthermore, I have made statements in several of my papers to show clearly that I have always assumed that continuous illumination may act as a stimulus. For example:

('10, p. 132) "This seems to indicate that the stimulus which regulates the rate of locomotion is dependent upon the amount of energy received, that is, *constant intensity*, not change of intensity."

('14 a, p. 653) "These results would be very serious indeed for the change-of-intensity theory if this theory maintained that light acts only in producing shock-reactions. I am, however, not aware that anyone ever held such a view. Light, as I have repeatedly stated (1907 and 1911) in all probability has an effect on physiological processes (activity etc.) in organisms, an effect which bears a definite relation with the amount of energy received."

('14 a, p. 656) "I have never maintained that the stimulating agent does not function in the process of orientation in animals in accord with the continuous action theory. In fact I believe that it does so function in some animals."

('16, p. 13) "Mast ('11, p. 163) reached conclusions regarding the orientation of *Eudendrium* which are in harmony with the work just mentioned [i.e., the continuous action theory of orientation]."

Regarding the nature of the electric stimulus I never expressed in writing an opinion of any kind. In view of all this evidence to the contrary it seems strange that anyone should accuse Jennings and myself of having "the *a priori* conviction that only a rapid change in the intensity of a galvanic current or of light is a stimulus."

cumulation of this substance is not proportional to the amount of light-energy received by the receptors, for the required amount is not accumulated, no matter how much or how little light may be received, if the change in intensity is not sufficiently rapid. The fact that the required amount of this substance is not accumulated if the intensity is slowly changed, indicates that it is removed either by further chemical changes, crystallization, transportation or adsorption, at a rate which prevents its accumulation in sufficient quantity to induce a response.

There are, however, also reactions of the 'all or none' type, which are within wide ranges independent of the time-rate of change in the intensity of the stimulating agent. In these, just as in the former, there is a definite threshold. A considerable amount of light-energy must be received by the receptors before there is a reaction, but the amount necessary to induce the reactions is within certain limits constant regardless as to whether it is received rapidly or slowly. This indicates that an accumulation of an appreciable amount of a specific photochemical substance is necessary to induce the reaction, and that this substance is, during the process of formation only slowly removed, if at all. Results obtained by Ewald ('13 and '14) in observations on the photic reactions of the eye of *Daphnia*; by Hecht ('18 and '19) in observations on *Ciona* and *Mya*; by Obreshkova ('21) on tadpoles; by Folger ('22) on *Amoeba* and by the writer on *Volvox* (not yet published) indicate that some of the responses in all of these forms are of this type. Such responses probably occur in many other forms.

There are numerous responses which proceed without any change in the illumination of the field. Many of these are, however, like some of those mentioned above, dependent upon the time-rate of change in the intensity of the light received by the receptors, such changes being brought about by changes in the position of the organism, of such a nature that various surfaces are alternately turned toward and from the source of light. Owing to this the illumination of the sensitive elements in the organism may change from practically zero to thousands of meter-candles without any change in the illumina-

tion of the field, resulting in reactions of the 'all or none' type. Changes of this sort may even occur in the absence of postural changes in the organism, because of internal movements, protoplasmic streaming, movement of pigment granules, and the like. It is, therefore, evident that it is very difficult to exclude the possibility of changes in the intensity of the light on the sensitive tissue.

There are, however, responses in which such changes appear to be absent, responses which continue indefinitely and in which the agent probably acts continuously; for example, certain reactions to constant electric currents, and some responses to light and temperature, especially those which involve irreversible changes in growth or chemical constitution. Such reactions are probably found in the orienting responses of plants and certain animals, e.g., the bending of plumules and tubeworms toward the light and the bending of roots toward the center of the earth. It is maintained by some investigators that there is nothing in the nature of an observable threshold in these responses. Arisz, for example ('11 and '15), holds that, in the plumules of *Avena*, it is impossible to ascertain when the response begins, but he is inclined to think that it begins immediately after the application of the stimulus. However this may be theoretically it is evident that there must be, as the amount of light decreases, a point below which there is no effect. If this is true, there is with reference to threshold no fundamental difference between various responses, for in all of them a certain amount of stimulating energy is necessary. In some this amount is so large that it can readily be measured, while in others it is so small that it is not perceptible.

Parker ('03), Bohn ('09), Loeb ('12), Minnich ('19), Garrey ('18), and others, maintain that photic orientation in insects is independent upon continuous illumination of the photoreceptors and continuous action of light. They contend that the circus movement observed in insects with one eye covered prove this. It has been maintained by some investigators, the writer ('11), and others, that the possibility of changes in the illumination of the sensitive elements of the retina have not

been excluded in any of the experiments in which circus movements have been observed, owing to the fact that extremely minute changes in the axial position of the eyes produce changes in the illumination of the retina if the light in all directions of space is not equal.

In attempting to meet this criticism Holmes and McGraw ('13) held a butterfly by the wings over a circular disk in such a way that when it attempted to turn in one direction the action of the legs caused the disk to rotate in the opposite direction. They maintain that when the insect was laterally illuminated with light of constant intensity while it was held thus, it attempted to turn continuously toward the light, and they consequently conclude that light acts continuously in the process of orientation.

Dolley ('16) repeated the experiments of Holmes and McGraw with some modification and confirmed their results, but he found that while the insect is attempting to turn toward the light it continuously moves its head up and down, and he maintains that this results in changes in the illumination of the sensitive elements of the retina and that the conclusion of Holmes and McGraw is consequently not warranted.

Holmes ('17) repeated the experiments, but he now fastened the head of the insect so that it could not move. He maintains that under these conditions the insect still attempted to turn toward the light, and he concluded as follows (p. 69): "Inasmuch as the conditions of the experiment were such as hardly to leave any room for fluctuations of light intensity to play any part, the only reasonable interpretation of the results is that they are due to the continuous action of the light." He does not, however, exclude the possibility that the action of the insect in every case may have been due to the increase in illumination produced by turning the light on or by changing it from one side to the other, for he does not state how long the action continued after the light was turned on. But even if the observed action was not dependent upon changes in the intensity of the light (I am inclined to think that Holmes is correct in his contention that it was not) does this warrant the conclusion that such reactions are due to the continuous action of the light?

In non-reversible reactions, such as the bending of a plumule toward the light, it is not difficult to conceive how a stimulating agent can act continuously, for in such reactions there is no return to the original condition, and consequently there is no time needed for restitution. But in a system in which the reactions are periodic or reversible, as they are in the muscular and nervous activity involved in the process of orientation in insects, the matter is quite different. In such systems there is after every action a return toward the original condition. Take, e.g., the action of the muscles in walking or flying, or the transmission of impulses in a nerve fibre. After the contraction of a muscle or the passage of an impulse the system undoubtedly returns toward the original state. The same is doubtless true for the photochemical changes that take place in the receptors. In all of these systems there is after each action a process of restitution, and during the period required for this process it is impossible to see how the stimulating agent can act. It is, therefore, evident that continuous action of the stimulating agent in the systems involved in the process of orientation in insects does not seem possible, and the results of recent experiments made by Dolley indicate that it does not obtain.

Dolley ('20) in some extensive and carefully controlled observations on the butterfly, *Vanessa antiopa*, found that the stimulating efficiency of light is, under certain conditions, higher if it is applied periodically than if it is applied continuously; that is, that a given amount of light-energy has a greater orienting effect if it is intermittent than the same amount has if it is constant. He found that at a flash-frequency of about 40 per second or higher, intermittent has practically the same stimulating efficiency as continuous light, that as the flash-frequency decreases the stimulating efficiency increases to a maximum (considerably higher than that of continuous illumination) at about 20 per second; after which it decreases, becoming approximately equal to continuous illumination at 10 per second and less at lower flash-frequencies. He found, moreover, that the stimulating efficiency depends upon the relation be-

tween the length of the light and dark periods in intermittent illumination: that at 20 or 30 flashed per second, e.g., intermittent illumination with the dark periods three times as long as the light periods, has a higher stimulating efficiency than it has with the dark and the light periods equal, and that the stimulating efficiency is still less if the dark periods are three times as long as the light. Similar results have recently been obtained in experiments on a tachina fly (Dolley, '21).

What do these facts signify? They seem to indicate that when a receptor is continuously illuminated it is not continuously stimulated; that there are periods during which the light acts, followed by periods during which it does not; sensitive periods followed by nonsensitive (refractory) periods; periods in which light induces, in the receptors, photochemical changes in one direction followed by periods in which the reverse (restitution) occurs; so that, when in intermittent illumination the light and the dark periods harmonize with the sensitive and the refractory periods, respectively, all of the light received is used in stimulation while in continuous illumination that received during the refractory periods is not used. If this is true, we would expect the stimulating efficiency of intermittent light of certain flash-frequencies to be higher than that of continuous light, just as observed by Dolley, and we would expect it to be of the 'all or none' type, light inducing in the photo-receptors the formation of a certain substance and that in turn producing an impulse as soon as a given amount has accumulated.

Dolley found, as previously stated, that the maximum efficiency for *Vanessa* is at approximately 20 flashes per second at room temperature. If the hypothesis presented above is correct, then in these insects stimulated by continuous illumination, at room temperature, approximately 20 impulses per second pass through each nerve element to the muscles. There is much evidence indicating that this is about the number of impulses per second that would pass, at room temperature, to the skeletal muscles in man during the period of contraction, especially in voluntary action.

The idea that the action of continuous illumination is periodic as set forth above, is in harmony with all of the results obtained by Dolley on the relation between flash-frequency and stimulating efficiency. This interrelationship is diagrammatically illustrated in table 1. In this illustration it is assumed that the maximum stimulating efficiency of intermittent light is at 20 flashes per second, and that at this flash-frequency all of the light-energy is used in stimulation. At 20 flashes per second the light periods are consequently represented as coinciding with the sensitive periods and the dark periods with the refractory periods; indicating that, in the time represented, there were sixteen units of energy, all of which were used in stimulation, while of the same amount of energy received in continuous illumination, represented in the first line, and in intermittent light of 80, 40 and 10 flashes per second, respectively, represented in other lines, only eight units were used, the other eight units being received during the refractory periods. The stimulating efficiency of intermittent light with a flash-frequency of 80, 40 or 10 per second ought, therefore, to be equal to that of continuous light, while that with a flash frequency of 20 per second ought to be higher, and this is precisely what Dolley observed.

In reference to intermittent light with flash-frequencies of 15, 25, 30, etc., the results obtained do not fit so perfectly into the scheme under consideration. All of them can, however, be made to harmonize with it if certain assumptions are permitted. Take, for example, intermittent light with a flash-frequency of 30 per second. This has an observed stimulating efficiency greater than continuous illumination, but less than intermittent light with a flash-frequency of 20 per second. This can be accounted for if it is assumed that restitution during the dark periods, owing to lack of time, is incomplete, and that this causes a decrease in the stimulating efficiency of the energy received during the succeeding light periods, thus making the stimulating efficiency as a whole less than that of illumination with a flash-frequency of 20 per second, and greater than that of continuous illumination. (This assumption is in harmony

TABLE I  
*Effect of flash-frequency on stimulating efficiency. Blank spaces represent light periods, black spaces dark periods; x, units of light energy*

Illu- mina- tion	Sensi- tive period	Refrac- tory period	Sensi- tive period	Refrac- tory period	Sensi- tive period	Refrac- tory period	Sensi- tive period	Refrac- tory period	Total Energy	Energy receiv- ed dur- ing sen- sitive periods	Observ- ed stim- ulating sen- sitive energy
Con- tinu- ous	x	x	x	x	x	x	x	x	16x	8x	y
Inter- mittent	x	x	x	x	x	x	x	x	16x	8x	y
40	2x	x	x	x	x	x	x	x	16x	8x	y
30	2 $\frac{2}{3}$ x	x	x	x	x	x	x	x	16x	8x	y +
20	4x	x	x	x	x	x	x	x	16x	16x	y + +
15	4x	$\frac{4}{3}$ x	x	x	x	x	x	x	16x	8x	y +
10	4x	4x	x	x	x	x	x	x	16x	8x	y
5	4x	4x	4x	4x	x	x	x	x	16x	8x	y -

with results obtained in direct electrical stimulation of nerves. (Schäfer, p. 475.) The observed stimulating efficiencies of all other flash-frequencies between 20 and 40 per second can be explained in the same way. Those observed in flash-frequencies below 20 per second are readily accounted for on the assumption that the flashes are longer than the sensitive periods and consequently extend over into the refractory periods, so that a portion of the energy received during each light period is not used in stimulation. This is clearly indicated in the illustration of the conditions which obtain in intermittent light with a flash-frequency of 15 per second.

The hypothesis presented is, consequently, in accord with the facts at hand. Further investigation will, however, doubtless make certain modifications necessary; for example, according to the hypothesis as illustrated the stimulating efficiency of light with 20 flashes per second should be two times as great as that of light with 40 flashes per second. The results at hand show that it is greater in the former than in the latter, but they do not indicate how much greater, and it is altogether probable that the ratio between the efficiencies of the two is not in exact accord with that demanded by the hypothesis. Furthermore, we have assumed that there is an instantaneous passage from the sensitive to the refractory period, whereas there is, in all probability, a gradual transition. We have, moreover, assumed that the sensitive and the refractory periods are equal. Some results obtained by Dolley ('20) indicate as previously stated, that this is not necessarily true. He found that intermittent light with 20 or 30 flashes per second has a higher stimulating efficiency if the dark period is three times as long as the light period, than it does if the dark period is equal to the light period, and that it has a higher efficiency if the two periods are equal than it does if the light period is three times as long as the dark. Consequently, if a receptor receives a given amount of light-energy in a given time followed by a dark period as long, the stimulating effect is greater than it is if the receptors receive the same amount of light energy in a longer time followed by a shorter dark period. In lower flash-frequencies it was found,

TABLE 2  
Effect on stimulating efficiency, of the ratio between the dark and the light periods in intermittent illumination. Blank spaces represent light periods, black spaces dark periods: 3 to 1, light period three times as long as dark period; 1 to 1, light period equal to dark period; 1 to 3, dark period three times as long as light period; x, units of light-energy

Illu- mina- tion	Sensi- tive period	Refrac- tory period	Sensi- tive period	Refrac- tory period	Sensi- tive period	Refrac- tory period	Sensi- tive period	Refrac- tory period	Energy receiv- ed	Observ- ed stim- ulating effici- ency
Con- tinu- ous	x x	x x	x x	x x	x x	x x	x x	x x	16x	y
20 flashes per sec.	3 to 1	2 2/3 x	4/3x						16x	y+
	1 to 1	4x							16x	y++
	1 to 3	4x							16x	y+++
10 flashes per sec.	3 to 1	2 2/3x	2 2/3x	2 2/3x					16x	y
	1 to 1	4x	4x						16x	y-
	1 to 3	8x							16x	y

however, that the shorter the dark periods the higher the stimulating efficiency (table 2). The fact that at certain flash-frequencies the stimulating efficiency is at a maximum when the dark period is longer than the light period, indicates clearly that the refractory period is longer than the sensitive period. The significance of the apparently contradictory results obtained with lower flash-frequencies is not clear, although they do not appear necessarily to be in opposition to the conclusion reached, for with them both the dark and light periods were so long that they probably extended, in every case, beyond the limits of the sensitive and the refractory periods, and the effect of this is entirely unknown.

We may, then, in so far as light is concerned, divide the stimuli (processes in the receptors) into two classes: one in which the processes are periodic and reversible and one in which they are continuous and irreversible. The former may again be divided into two classes: one in which the reactions are dependent upon the time-rate of change in the intensity of the stimulating agent and one in which they are not.

A great majority of the reactions to light in animals belong to the periodic class. There are, however, some which appear to belong to the continuous class, e.g., the bending of *Eudendrium* toward the light and the increase in activity in many forms in the presence of light. But it must be admitted that we are still on doubtful ground here. In the reactions which belong to the continuous class there is no perceptible threshold; the amount of stimulating energy necessary to initiate a response is very small; the response, as previously stated, appears to be irreversible; it consists in movements in a given direction without a return; and the magnitude of it is probably specially related to the stimulating agent, although this has not as yet been definitely established in any case.

Among the reactions to light in plants there are probably relatively many more that belong to the continuous class than there are among the reactions to light in animals, but here, too, the problem has not been investigated thoroughly enough to admit of very positive statement.

## THE BUNSEN-ROSCOE LAW

According to the Bunsen-Roscoe law, the effect of a given amount of energy is constant no matter how the two factors involved may vary, i.e., a strong light acting a short time has the same effect as a weak light acting a long time provided the energy received is the same. But if the amount of energy varies, then the effect varies in direct proportion with it, e.g., if the intensity remains constant and the period of illumination is doubled or if the period of illumination remains constant and the intensity is doubled, the effect is doubled.

Ewald ('13) Patten ('14, p. 270), Loeb ('18, p. 18), and others contend that the Bunsen-Roscoe law holds for all reactions in which intermittent light of a given average intensity has the same stimulating effect as continuous light of the same intensity.<sup>11</sup> If this is true, and it doubtless is in certain respects, then there are no reactions for which the Bunsen-Roscoe law does not hold, for it is evident that if the flash-frequency of intermittent light is sufficiently high it becomes continuous, just as the circumference of a circle becomes straight when it is sufficiently large. Intermittent light, therefore, has in *all*

<sup>11</sup> Loeb says ('18, p. 99): "Mast has recently published experiments on the relative efficiency of the various parts of the spectrum by a method based on the assumption of the validity of the Bunsen-Roscoe law for the heliotropic orientation of these organisms. If his assumption is correct, it contradicts the theory which Jennings and Mast have defended now for more than fifteen years; if his assumption is wrong, his experiments on the relative efficiency of various parts of the spectrum cannot be correct. . . . He does not seem to have noticed that his method was based on this assumption."

In the experiments referred to by Loeb I compared the stimulating effect of light in different regions of the spectrum with that of intermittent light of very high flash-frequency. By referring to the section on methods and materials in my paper ('17, p. 483) it can be seen that the validity of my results is entirely independent of the character and composition of the white light used provided only that these do not change. They would, in my opinion, be quite as valid if the Bunsen-Roscoe law or even Talbot's law did not apply to the orienting reactions of the organisms investigated as they would if it did, and the assumptions made have, as far as I can see, absolutely no bearing on any "theory which Jennings and Mast have defended." They certainly do not invalidate the idea that there are orienting responses which are dependent upon the time-rate of change of luminous intensity on the photosensitive tissue.

*reactions*, those which are dependent upon the time-rate of change in intensity as well as those which are not, the same stimulating efficiency as continuous light provided the flash-frequency is sufficiently high. For the human eye the flash-frequency necessary varies from about 10 per second in very low illumination to about 60 per second in very high illumination, and it is approximately the same for the eye of the fish (Mast, '16, p. 220) and probably also for that of the butterfly (Dolley, '20). If the flash-frequency is lower than this, intermittent light does not have the same stimulating efficiency as continuous light in any of these cases, and it is obvious that if it is sufficiently low it will not have the same stimulating efficiency in any reactions in any organisms. It is consequently evident that there are no reactions to light which are not in accord with the Bunsen-Roscoe law in certain respects and that there are none which are in accord with this law in all respects. The statement, then so frequently made without qualification that this or that reaction is in accord with the Bunsen-Roscoe law is meaningless; and the attempt made by a number of investigators to use the applicability of this law without qualifications, as a criterion of 'tropism' is futile.

It has been demonstrated for a number of different organisms, *Avena*, *Ameba*, *Volvox*, *Eudendrium*, *Ciona*, *Mya*, *Daphnia*, *Rana* (tadpoles), and others, that the amount of light-energy necessary to induce certain reactions is over considerable ranges of intensity nearly constant for each species. This indicates that within certain limits the Bunsen-Roscoe law holds approximately in reference to the initiation, the threshold, in all of these reactions, but that it does not hold even approximately in any of them beyond these limits of intensity. In the intensities beyond these limits, as well as in those within them, intermittent illumination has, however, the same stimulating efficiency as continuous illumination, provided the flash-frequency is sufficiently high. Consequently, while the Bunsen-Roscoe law does not hold beyond certain limits of intensity in reference to the threshold as a whole, it still holds for shorter periods.

In none of these cases is there, however, any evidence showing that the effect varies in direct proportion to the amount of energy received. In this respect there is no evidence indicating that the Bunsen-Roscoe law holds in any reactions, although it is not unlikely that such evidence may be forthcoming. It is well known that the magnitude of many reactions depends upon the amount of stimulating energy received by the receptors, but the relation between these phenomena is so complicated and variable that it has so far been impossible to state it in the form of a law. Garrey ('18, p. 121) implies that in the robbery-fly with one eye covered deflection toward the functional eye bears a rather definite relation to the intensity of the light such that the insects under these conditions serve a 'a crude photometer.' He did not, however, carry the investigations much beyond the qualitative stage.

It may be concluded, then, that the evidence at hand indicates that the Bunsen-Roscoe law holds in certain respects for all reactions provided the time factor is sufficiently short and that it does not hold for any reactions in any respect if the time factor is sufficiently long; that it holds approximately, within certain limits of variation in intensity, in reference to the amount of energy required to initiate reactions (threshold) but that it does not hold for any reactions in reference to the relation between the stimulating energy and the magnitude of the reaction.

#### DISCUSSION

Much of the controversy concerning the process of orientation in organisms has its origin, I believe, in the tendency to refer, in discussing the subject, to theories of orientation or 'tropisms' rather than to the various factors involved in the theories, to misconceptions regarding the factors implied in the various theories and to lack of precision and consistency in the definition of the various theories. For example, Loeb says ('18, p. 15): "The forced orientations of plants by outside sources of energy had been called tropisms; and the theory of animal conduct based on the symmetrical structure of their body was, therefore, designated as the *tropism theory of animal conduct*." He

thus on page 15 restricts the term 'tropism' or orientations which are directly dependent upon 'outside sources of energy' and 'the symmetrical structure of the body.' But as he proceeds he broadens the definition until at the close of the book (p. 172) he includes all reactions in all organisms, even voluntary actions in man. And yet he repeatedly refers to 'the tropism theory' (pp. 93, 156, 163, etc., etc.). In the presence of so many different sorts of reactions, all designated 'tropisms,' one is at a loss to know which exemplify "the tropism theory." And, indeed, there is evidence indicating that Loeb himself is at times not perfectly clear in reference to this. In one place ('18, p. 143) he refers to the aggregating reactions by trial and error or random movement in *Paramecia* as 'tropisms'; in another (p. 145) he says Jennings is probably correct in maintaining that they are not 'tropisms.' In one place ('12, p. 40) he says that reactions which carry an organism toward one of two sources of stimulation are not 'tropisms'; in another ('18, pp. 156-163) he implies that they are. In one place ('18, p. 15) he contends that only those reactions which are dependent upon the direct action of the environment and bilateral symmetry are 'tropisms'; in another (p. 172) he implies that neither of these factors are essential for he says, "The persistent courtship of a human male for a definite individual female may appear as an example of persistent will, yet it is a complicated tropism in which sex-hormones and definite memory images are the determining factors." In this phenomenon it is evident that symmetry, at least, is not essential for it occurs in asymmetrical individuals. The loss of a leg, an eye, an arm, an ear, does not prevent courtship!

It is consequently impossible to ascertain precisely what Loeb means by the phrase 'the tropism theory,' and the same may be said in reference to other theories of orientation. Concerning the factors involved in some of these theories it is, however, not difficult to get a clear conception.

The first explanation of orientation in organisms that I have discovered was given by Ray in 1693. Ray maintains that photic orientation in plants is produced by difference in the

rate of growth on opposite sides of the responding structure. He contends that plants growing near a window, bend toward the light because the side facing the window is cooler than the opposite side, and consequently grows more slowly. De Candolle ('32) gives precisely the same explanation except that he maintains that the difference in the rate of growth on opposite sides is due to difference in illumination on opposite sides instead of difference in temperature. According to these explanations the following factors are involved in orientation: 1) Orientation is dependent upon the relation of the intensity of the stimulating agent on opposite sides of the reacting structure. 2) It is the result of the balanced effect of the reaction of similar tissues symmetrically located on opposite sides of the orienting structures. 3) The responding tissues are stimulated directly. There is no division into receptor and motor tissue, no transmission of impulses. 4) The response and the stimulus are specifically related in magnitude. 5) The stimulating agent acts continuously. It acts after the structure has become oriented as well as during the process of orientation.

Other factors which have been suggested by various authors as important in the process of orientation may be presented as follows: Ray-direction (Sachs, '76); Changes of intensity (Darwin, '80); Volition (Romanes, '83); Muscle-tonus, Memory-images, Sex-hormones (Loeb, '97, '18); Random movements or 'trial and error' (Jennings, '06); Differential responses to localized stimulations (Mast, '11, '12); Molecular polarization (Bohn, '20).

Some of these factors have never been applied to orientation in insects, e.g., 'trial and error,' which in certain quarters has been so frequently ruthlessly torn from positions it never occupied. This factor is under certain conditions of great importance in the process of orientation in flatworms, earthworms, fly-larvae, certain molluscs (Copeland, '18) and other organisms, but as far as I know, it has never been maintained, except in reference to phylogenetic origin, that it is of any considerable importance in the process of orientation in insects. Years ago certain investigators, Romanes, Graber, Lubbock, and others,

strongly emphasized volition as a factor in orientation of insects. This contention, although, in my opinion, by no means positively excluded, has not been emphasized in recent years by any one of note. Bouvier ('18) says: "The old anthropocentric school has passed away; we no longer attempt to understand insects by comparing them to men." It is, however, nevertheless, still vigorously attacked in unmistakable terms (Loeb, '18, and Bohn, '20).

At present the Ray-Verworn or De Candolle-Verworn theory as modified by Loeb and others is widely accepted as offering a satisfactory explanation of orientation in insects. This theory, in its modified form consists of five of the factors mentioned above, symmetry, difference of intensity, continuous action, tonus, and direct proportionality between stimulus and reaction. To what extent are these factors actually involved in the process of orientation in insects?

It is self-evident that if the muscles in the locomotor appendages on opposite sides of a bilaterally symmetrical organism have the same tonus and work alike, it will take a direct course and that if they do not have the same tonus and do not work alike it will turn. No experimental evidence is needed to demonstrate this. It is also well known that if certain receptors symmetrically situated on opposite sides, are unequally illuminated some insects tend to turn, whereas if the receptors are equally illuminated, they tend to take a straight course. I should like to emphasize in this connection the interesting observations of Patten on the scorpion ('17). It has, moreover, been fairly clearly established that the degree of turning depends, under certain conditions, upon the extent of the difference in the intensity of the illumination of the receptors on opposite sides. Furthermore, it has been fairly conclusively demonstrated that the orienting reactions are not dependent upon a change in the illumination of the receptors and that the tonus of certain muscles in the legs is, at least under certain conditions, dependent upon the illumination of the receptors. These facts have been offered by various investigators in support of the five factors which constitute the theory under consideration and they

are, in my opinion, the only facts which lend support to this theory.<sup>12</sup> Do they actually establish it?

A. There is no evidence indicating that orienting reactions are in magnitude proportional to the stimulus. It has, however, been demonstrated that in certain organisms it requires a given amount of energy to initiate certain reactions involved in orientation. This probably holds for insects, although it has not as yet been demonstrated for these forms. Thus it may be said that if there is a proportional relation between stimulus and response it refers only to the initiation of the response, the threshold.

B. The work of Holmes and his students seems to demonstrate that the orienting stimulus in certain insects is not dependent upon changes of intensity on the sensitive tissue. Thus it appears that the continuous action factor holds for orientation in some insects in a certain sense. The results obtained by Dolley seem to show, however, that while orienting stimulation in these insects is not dependent upon change of intensity, the action of the stimulating agent is not continuous. If this is true, it must be admitted that in the strict sense of the word the continuous action factor does not hold.

C. The remaining three factors, symmetry, difference of intensity and tonus, in so far as they refer to orientation, all center around the idea that orientation is the result of some

<sup>12</sup> Galvanic orientation in various organisms belonging to widely separated groups, e.g., *Paramecium*, *Volvox*, *Paleomonetes*, and amphibian tadpoles, is in certain respects in harmony with this theory. It is often maintained that the process of photic orientation is identical with that of galvanic orientation and that consequently it must also be in harmony with the theory under consideration. This supposed identity has, however, in my opinion, not been firmly established even in a single case. In those usually cited in this connection, *Paleomonetes* and *Volvox*, the evidence in support of it is certainly extremely weak. In *Volvox* orientation in light is probably due to an increase in the effect of the stroke of the flagella on the side of the colony furthest from the source of stimulation (Mast, '07); in the electric current it is due to a decrease on the opposite side (Baneroff, '07). In *Paleomonetes* orientation in the electric current appears to depend upon a direct effect of the stimulating agent either on the muscles or on the nervous system; in orientation in light there is no evidence indicating a direct effect of the stimulating agent and the process appears to be fundamentally different from that which obtains in galvanic orientation.

sort of a balance between the action of symmetrically located receptors and appendages on opposite sides of the body, the organs on one side working against those on the other in such a way that equality in action results in a straight course, and inequality in turning.

Some of the facts presented above support this idea but there are many others which are not in accord with it, for example, the following:

1. Certain organisms, e.g., toads (Mast, '11), bumble-bees (Dolley, personal communication), and caterpillars (Buddenbrock, '17) when exposed to light from two or more sources go directly toward one. Others, crabs (Holmes, '08), go sidewise toward the light. The receptors are consequently not equally illuminated in any of these organisms when they are oriented.

2. Some forms, e.g., *Caprella* (Mast, '11) and insects on the wing turn at times toward the dorsal or the ventral surface when they orient. These two surfaces are not symmetrical. Orientation in reference to them can, therefore, not be dependent upon balanced action in receptors and appendages.

3. Fire-flies (Mast, '12) after momentary asymmetrical stimulation turn through the proper angle and proceed, in total darkness, directly toward the source of illumination. The retention of orientation in these insects is, therefore, not due to continuous equal action in the receptors on opposite sides.

4. Insects with one eye covered or with the front and the middle legs on one side removed orient fairly accurately under certain conditions. Equal action in receptors and appendages on opposite sides is impossible in these.

5. Robber-flies under certain conditions, while they are strongly tilted in one direction, turn toward the light in the opposite direction, that is, in the direction contrary to that demanded by the tonus hypothesis as formulated by Garrey.

6. In orientation in asymmetrical forms, e.g., tadpoles of *Amaroucium* (Mast, '21) and in some symmetrical forms which have but a single median receptor, e.g., certain snails (Copeland, '18), none of the factors in the theory in question apply without modification.

7. When insects are oriented in light from two or more sources differing in intensity, distance or size, the eyes on opposite sides are not equally illuminated. Consequently, when an insect is oriented out of doors under normal conditions, the two eyes are rarely if ever equally illuminated.

These facts demonstrate conclusively, I believe, that symmetry has no fundamental significance in the process of orientation in insects, that orientation is not primarily dependent upon anything in the nature of a balance in the action of organs of any kind, symmetrically located on opposite sides. There are, then, numerous facts which are not accounted for by any of the factors found in the Ray-Verworn theory as modified by Loeb and others. Any theory of orientation worthy of consideration should, it seems to me, account for these facts.

In my investigation on the fire-fly ('12) I became convinced that orientation in these insects is the result of series of reflexes, the nature of which is dependent upon the localization of the stimulus in the eye. In this investigation it was found that the female in response to a flash of light produced by the male, turns the ventral surface of her abdomen toward him no matter where he may be located; and that the male, in response to a flash of light produced by the female, turns and flies or walks directly toward her no matter where she may be located. In both the response is carried out after the flash which induced it has disappeared. In both the extent and the direction of turning, i.e., the character of the reaction, depends upon the location on the retina of the image produced by the flash. If it is near the posterior surface of the eye the response is markedly different from what it is if it is near the anterior surface or near the dorsal or the ventral surface. The response is not dependent upon anything in the nature of balanced stimulation: for, in many situations, only one eye is stimulated. In this form then orientation is clearly dependent upon the localization of the stimulus. Stimulation of a given point in the retina of either eye sets up a series of coördinated responses in both wings or in all of the legs, the nature and the extent of which depends specifically upon the location of the point stimulated, just as

the nature of scratch reflexes in a dog or a cat depends upon the localization of the stimulus. The orienting responses in the fire-fly are in fact in many respects similar to scratch reflexes. They are actual reactions of the 'all or none' type, not merely tonus effects. Not one of the factors in the Ray-Verworn theory as modified by Loeb, Garrey, and others, presented above, are essential: neither symmetry, nor difference of intensity, nor continuous action, nor tonus, nor proportionality between stimulus and response. Localization of the stimulus and the structure (anatomical, histological, and physiological) of the reacting system are the essentials in the process, a structure which is rooted in the experiences of the organism and its ancestors.

Taliaferro ('20) in observations on *Planaria* indicates that the explanation given above for orientation in the fire-fly holds also in its essential features for this form. In specimens with one eye removed, in which photic orientation is fairly accurate, he found that if the stimulus is located in the anterior portion of either eye the animal invariably turns away from the side stimulated, but that if it is located in the posterior or the ventral portion it invariably turns toward this side. The orienting response in this form is, consequently, clearly dependent upon the localization of the stimulus. He also found that only those rays which enter the eye parallel with the longitudinal axis of the rhabdomes, or nearly so, are effective in stimulation. Moreover, Buddenbrock ('19, p. 340) maintains that localization of the stimulus in the eye is a predominant factor in orientation in gastropods. And Rádl ('03, p. 107) has expressed similar ideas in reference to insects.

Is the effect of localization of the stimulus essential in the process of photic orientation in insects in general, as it is in fire-flies, and if so what other factors are involved? First of all it should be borne in mind that insect eyes are image-forming eyes. Consequently, when an insect is exposed to a source of light which illuminates a large portion of the surface of the eyes there may be, owing to the formation of an image, only a minute portion of the retina illuminated, and the stimulus may consequently be confined to a very small region in the eye.

Now, as we have pointed out in the preceding pages, if the stimulus is confined to the retina of one eye and is located in the lateral or the posterior portion, the insects studied (*Eristalis* and *Erax*) turn toward the side stimulated, just as the male fire-fly does; but if the stimulus is only momentary they turn only through a very short distance. They do not turn far enough to become directed toward the source of stimulation as does the fire-fly. However, there is clearly a response in all of the legs, and the character of this response depends upon the localization of the stimulus. If the stimulus is located at the posterior edge of the retina, the legs on the side stimulated move backward while those on the opposite side move forward. If it is near the anterior edge this does not occur. In general the legs on both sides move in a coördinated way, in such a direction as to tend to turn the anterior end of the animal toward the source of stimulation. If the image is located at the posterior edge of the retina and the insect turns, the image travels forward; so that one point after another on the retina is stimulated, the stimulation of each point resulting in responses, which produce a slight turn toward the light. Thus, owing to the stimulation of successive points on the retina, the insect turns until the image reaches the anterior part of the eye where it no longer induces turning. In some respects these orienting responses are, in principle, like those in the fire-fly. In both there are series of reflexes dependent upon the localization of the stimulus. In the one these reflexes are, however, in magnitude less specifically related to the localization than in the other. In the fire-fly momentary stimulation of a given spot near the posterior surface of the eye causes turning through an angle of  $90^\circ$  or more, in other insects momentary stimulation of the same relative spot causes turning through only a very small fraction of  $90^\circ$ . In both there is no tendency to turn when the stimulus is located in a certain region in the anterior portion of the eye. In the latter this holds regardless as to whether one or both eyes are stimulated. Whether or not this also holds for the fire-fly is not known.

All of what has been said refers to stimulation of one eye alone. If both eyes are simultaneously stimulated the responses are modified, but this does not militate against the principle of differential response to localized stimulation. If the same region in both eyes is simultaneously and equally stimulated, the insects do not turn and there is no indication of a spreading of the legs as if those on one side, in response to the stimulation in one eye tended to turn in one direction while those on the opposite side, in response to the stimulation in the other eye, tended to turn in the opposite direction; nor is there any indication of greater activity on both sides. It must, consequently, be assumed that stimulation in one eye is totally inhibited by equal stimulation of the same region in the other eye. Moreover, it must be assumed that the response (reflexes) induced by the stimulation of a given region in the retina of one eye are modified or inhibited by simultaneous stimulation of different regions of the other eye, in such a way that a given stimulus in one eye is neutralized by a progressively weaker stimulus in the other eye, as one proceeds toward the posterior surface; for when an insect is oriented in light from two sources at the same distance but unequal in luminous intensity the two eyes do not receive the same amount of light energy. Under such conditions the weaker image in the one eye is farther back than the stronger in the other, and the greater the difference in the illumination received from the two sources the greater the difference in the location of the images in the two eyes (figs. 11 to 13). If, then, there is no turning effect in either direction when the insect is oriented and the effect of the stronger light in one eye is neutralized by the effect of the weaker in the other eye it must be because the weaker image is located farther back than the stronger. Whether or not there is anything in the nature of integration in this process, I am unable to say.

Similar effects have been observed by Baglione ('00), Tiedermann ('10), and Veszi ('11) in reflexes in the frog. These investigators found that in certain nerves there is no reaction if two sensory roots at the cord, either on the same side or on opposite sides, are simultaneously stimulated, but that there

is a reaction if either root is stimulated alone. The reaction normally produced in the frog by the stimulation of a nerve on one side may consequently, if the results obtained by these investigators are valid, be inhibited by simultaneous stimulation of the same nerve on the opposite side. Dunlap's discovery ('20) that "vision in one eye can be inhibited by stimulation of the other eye" and that this inhibition is dependent upon phenomena in the central nervous system also lends support to the view under consideration.

The preceding statements indicate that the turning or orienting effect of a spot of light on the retina decreases as it proceeds from the posterior edge of the eye forward. And the fact that insects with but one functional eye orient fairly accurately under certain conditions and proceed directly toward a source of light, indicates that the turning effect is zero when a certain part of the anterior portion of the retina is illuminated (fig. 13). If this is true then, when the part of the retina mentioned is simultaneously illuminated in both eyes, the absence of turning to the right or to the left is due not to inhibition, as it is when other parts of the retina are illuminated, but to the absence of stimulation which would induce turning. And if this holds it is evident that if the illumination of the part of the retina designated has any direct effect on the movement of the organism it induces responses which tend to carry it directly forward, i.e., directly toward the source of light. The experimental evidence at hand indicates, however, that the rate of locomotion in insects is not to any considerable extent, immediately dependent upon illumination. That is, the rate of locomotion is, within wide ranges, practically the same in different luminous intensities provided the insects are not subjected to these intensities for long periods. It must, therefore, be concluded that when an insect is oriented in light, the light has little or no immediate effect on locomotion, either in reference to direction or in reference to rate. If this actually proves to be true, and a stimulus is defined as something which produces either a change in direction or in rate of movement, it must be admitted that after an insect is oriented and is proceeding toward a source of light it is not appreciably stimulated by the light.

The explanation of orientation presented has no bearing on the question as to the nature of the stimulus. It would hold regardless as to whether the stimulus acts continuously or not, or as to whether it depends upon time-rate of change in the intensity of the agent or not. Nor does it have any bearing on the question as to the relation in magnitude between the stimulus and the response. The facts presented in the preceding pages indicate, however, that while the orienting stimulus is not dependent upon change of intensity of the stimulating agent on the receptors, it is not continuous. They indicate that it is of the 'all or none' type and that the orienting reaction is not proportional in magnitude to the stimulus, although it is dependent upon it. The initiation of the stimulus, however, in accord with the Bunsen-Rosecoe law, doubtless requires within certain limits, a given amount of energy regardless of the rate of its reception.

Balanced stimulation and balanced response in reference to symmetrical organs on opposite sides plays a rôle in photic orientation under normal conditions, but not in an antagonistic or competitive sense, as implied by the Ray-Verworn theory modified by Loeb, Garrey, and others; and the rôle that it does play is, in my opinion, superficial in importance, for insects with one eye covered or with legs on one side removed can still orient.

There are, according to the explanation presented in the foregoing pages, two essential factors involved in the process of orientation in insects: series of coördinated reflexes dependent upon the localization of the stimulus (differential response to localized stimulation) and inhibition. This explanation is, I believe, in harmony with all of the facts known in reference to the process of orientation in insects, and the factors involved are fundamental in the process of photic orientation in all other bilaterally symmetrical organisms with photoreceptors on opposite sides, and also in certain asymmetrical organisms with well differentiated eyes either compound or simple. However, in some bilaterally symmetrical forms, e.g., earthworms, flatworms and fly larvae, random movements (trial reactions)

play a predominant rôle and localization and inhibition are normally of little significance. In organisms with a single simple receptor, e.g., *Euglena*, inhibition does not apply and localization applies only in a limited sense, for the location of the stimulus is probably always the same. In the colonial forms, *Volvox*, e.g., there is no indication that inhibition is involved in orientation; and while there are numerous receptors, differential response to localized stimulation is probably of little significance, if it exists at all. In higher plants, according to the results obtained by Fitting ('07) the cells in the motor tissues act essentially alike, each motor cell responding individually in such a way as to tend to turn the entire organ involved toward or from the source of stimulation. If this is correct, orientation in these forms is not the result of a balance in the action of the cells symmetrically located on opposite sides of the reacting structure.

In some organisms the orienting stimulus depends upon the time-rate of change in the intensity of the stimulating agent; in others it does not. In some the stimulating agent acts only momentarily, initiating a series of responses which result in orientation without further stimulation; in others the stimulating agent acts intermittently and in still others probably continuously throughout the whole process.

It is not our purpose to present here an exhaustive analysis of the process of orientation in the various groups of organisms, but if we are correct in the statements made in reference to it, it is evident that it differs greatly in different organisms. Is there any indication that orientation is the same in origin and significance in all?

Concerning the question as to the origin and the biological significance of photic orientation, the question as to why organisms go toward or from the light, there is much diversity of opinion. The idea that the orienting reactions in insects are controlled by psychic phenomena, likes and dislikes, pain and pleasure and that their origin is similar to that of consciousness in man, has long since practically disappeared among biologists who now in the main champion one of two different views, the

one centering around the idea that the orienting reactions are fundamentally adaptive and originated like other adaptive characters; the other around the idea that they are fundamentally non-adaptive and originated accidentally. Darwin, Jennings, Buddenbrock, et al., are strong advocates of the former, while Loeb is usually looked upon as the chief exponent of the latter view.

Jennings ('06) maintains that the process of orientation differs in different animals, that it is indirect in some, being dependent upon random movements (trial and error reactions), while it is direct in others, there being no random movements involved. He holds that simple random reactions are more primitive than random reactions which result in orientation (indirect orientation), and that these are in turn more primitive than direct orienting reactions such as are found in the reactions of insects to light. He contends that the latter developed by the elimination of useless movements, owing to readier resolution of physiological states after experience. According to this view, then, direct orientation is a complex adaptive phenomenon dependent upon the experience of the ancestors of present species, and transmitted by inheritance.

Loeb maintains that orientation is essentially the same in all organisms, plants as well as animals, that it is among the simplest reactions, reactions in which the more complex responses have their origin and that it is directly related to the environment, like the orientation of a weather vane in the wind or iron filings in a magnetic field or Hammond's 'mechanical dog' in a beam of light. He contends that it is fundamentally non-adaptive, like the orienting reactions to electricity, which occur only under experimental conditions, that experience in the present individuals or their ancestors is not involved, and that learning and heredity are not involved. These views he supports in unmistakable terms, basing his contention largely upon the facts that insects make circus movements, that in their photopositive reactions they can be made to go from higher to lower intensities, that they often fly into a flame and are killed, that they sometimes lay eggs on substances which the larvae cannot eat, and similar well-known phenomena.

That there are useless non-adaptive orienting reactions to light in insects cannot be doubted, reactions which prove fatal in countless instances, but these reactions occur usually, if not always, under abnormal conditions and they consequently do not support the contention that photic orientation is fundamentally non-adaptive. One might as well contend that, if a wolf, owing to positive reactions to meat, is caught in a trap, positive reactions of wolves to meat are non-adaptive. The photopositive reactions of insects under normal conditions usually result in their escape from places of danger. They tend to preserve the individual and the race. They are, as a whole, adaptive and they doubtless originated in the same way as other adaptive reactions originated.

Jennings' view regarding the origin of direct orientation is supported by a mass of evidence ('06), and while its validity has not been absolutely demonstrated, it is in full accord with the facts as far as they are known. However, no matter whether this view is valid or not, if we are correct in the conclusion that photic orienting reactions in insects consist of series of reflexes specifically related to the localization of the stimulus, it is evident that the orienting reflexes must have been somehow established by a process analogous to learning, either directly or by the elimination of certain responses in accord with the theory of natural selection; for the stimulation of different regions of the eye induces different series of coördinated reflexes, each of such a nature as to turn the insect toward the light. And since photic orientation is apparently as perfect in the young individuals as it is in the old ones, the orienting reflexes must have been established in the ancestors of the present races and handed on by inheritance. In other words, photic orientation in insects is an instinct dependent upon a structure which is founded upon the experience of the race.

Moreover, photic orienting reactions in insects are specifically related to the future: Why does illumination of a given region of the retina at the anterior end of the eye induce one series of coördinated reactions in the locomotor appendages, while equal illumination of a region at the posterior end induces a different

series? The reactions under both conditions tend to carry the insect toward the light. They have in some way become so related to the localization of the stimulus that the end in view is attained. Orienting reactions in insects consequently have a future reference. They are in reality reactions to a sign just as is the reaction of a dog to the picking up of a stone or a stick. Insects, like men, live in reference to the future as well as in reference to the past and the present, and they can no more be fully understood without considering the future in the one than they can in the other.

I realize full well that these are teleological concepts and that teleology is in disrepute among biologists of the day, but I believe that this is due to a restricted view of biological processes and to the apprehended contradiction between teleology and mechanism. If teleological conceptions lead to a more comprehensive understanding of nature they should, of course, prevail regardless of their relation to mechanism. I believe, however, that teleology and mechanism are in perfect harmony, for I believe with Hume that the essence of cause is sequence and, consequently, that in a series of interrelated phenomena for which the doctrine of determinism holds, future events are logically just as truly the cause of present events as are those which have passed.

But how can this be? One phenomenon is said to be causally related to another if the one can not be omitted without preventing the occurrence of the other. Whether or not the omission of the one prevents the occurrence of the other can be ascertained only by observed repetition, e.g., if a dog runs every time a stone is picked up and does not respond in this way when it is not picked up, it is said that the running of the dog is caused by the picking up of the stone. This, and similar observations, it is maintained, show that the present is causally dependent upon the past; but have we not precisely the same reason for saying that it is causally dependent upon the future? If an inexperienced dog sees a man pick up a stone he does not run, but if the man hits the dog with the stone he runs; and if this is repeated a number of times the dog will run whenever he

sees a man pick up a stone. However, if the process is repeated a number of times without injuring the dog he will cease to run when he sees a man pick up a stone. This shows that the cause of the running reaction is an anticipated future phenomenon, for if this is omitted the reaction does not occur. The running reaction of the dog is consequently dependent upon future events in the same sense that it is upon past events. The response of the dog when he sees a man pick up a stone is related to what is likely to follow. He is consequently living and responding in reference to the future, and the same may be said regarding insects and various other organisms from *Ameba* to man, in which reactions to 'signs,' 'representative stimuli,' 'conditioned reflexes' occur. Certain reactions in all of these depend, not solely upon the present, but also upon the past and upon what may occur in the future. All of them have the faculty of binding time. If this is true Korzybski's ('21) contention that man is distinguished from other organisms by his 'time-binding' ability, applies only in reference to degree, if at all, for there are no organisms in which the reactions are solely dependent upon the present.

#### SUMMARY

*Eristalis* and *Erax* are photopositive on the wing as well as on foot. *Eristalis* orients exceptionally accurately especially when on foot. *Erax* does not orient very precisely. If laterally illuminated, both turn directly, without trial or random movements, toward the source of light.

In orienting on the wing *Eristalis* turns up or down as well as to the right or left. This probably holds also for *Erax*. It cannot be due to unequal illumination of the two eyes as the Ray-Verworm theory demands.

In light from two sources both go toward a point between the sources. The location of this point depends upon the relation in the luminous intensity of the light received from the two sources. The greater the difference the nearer the more intense illumination the point is located.

In flying specimens this holds no matter whether the two beams are in a horizontal, in a vertical, or in any other plane. This was observed in *Eristalis* only. It indicates that the orienting response depends upon the location of the stimulus in the eyes.

When *Eristalis* or *Erax* is oriented in light from two sources on a horizontal line the two eyes are not equally illuminated and they do not receive the same amount of light, except when the light received from the two sources is equal. Under natural environmental conditions the two eyes are consequently rarely if ever equally illuminated when these insects are oriented.

*Eristalis* and *Erax* with the front leg on one side removed orient nearly as precisely as normal specimens. If the direction of the rays is changed they turn toward the light either to the right or the left.

If the front and the middle legs on one side are removed, *Erax* usually goes fairly directly toward the light, but only for a short distance after which it usually deflects rather sharply toward the normal side; then it ordinarily attempts to turn in the opposite direction, but usually fails and falls over. The movements of the legs are not well coordinated. *Eristalis* with the front and the middle legs on one side removed walks much more freely than *Erax*. In a horizontal beam of light, it at first deflects strongly toward the normal side, but after a few days it orients fairly accurately. This shows that orientation is not necessarily dependent upon equal or balanced action in locomotor appendages on opposite sides in accord with the Ray-Verworn theory.

Specimens of *Eristalis* with the front and middle legs on one side removed and either eye covered may under certain conditions proceed fairly directly toward the light. If the direction of the rays is changed while they are thus proceeding toward the light, they reorient by turning either to the right or to the left, showing that the movements of the legs on either side may be controlled by impulses originating in either eye. No observations were made on *Erax* in reference to this.

If the front and the middle legs on one side are removed and the eye on the opposite side is covered, illumination of the postero-lateral surface of the functional eye induces backward movement of the legs on the normal side; illumination of the lateral surface, lateral movements; illumination of the anterior surface, forward movement; and illumination of the antero-median surface, forward movement toward the median line. This shows that the response depends in part upon the location of the stimulus in the eye, and not solely upon the magnitude of the stimulus.

If one eye is covered *Erax* leans or tilts toward the normal side. This is the result of flexure of the legs on the normal side and extension of those on the other side. The degree of leaning or tilting is greater in strong than in weak light, and it is greater on a white than on a black background. If the lower portion of one eye and the upper portion of the other are covered *Erax* leans toward the latter even if this eye receives less light than the other, i.e., it tilts toward the eye which receives least light. This indicates that tilting is largely dependent upon the illumination of the ventral surface of the eye and that it is more intimately correlated with the location of the stimulus in the eyes than with the relation in the amount of light received by them.

The tilted posture appears to be the result of an attempt to turn toward the light without moving the feet. It is held for long periods, even if the illumination which induced it is entirely eliminated. Consequently, if it is due to the effect of light on muscle-tonus, as Garrey maintains, continuous light is not necessary for the maintenance of the tonus.

When *Erax* is tilted the legs on the blind side are much more extended than those on the normal side, and the feet consequently tend to move faster on the former than on the latter side, resulting in a curved course (*circus* movements) as Garrey maintains; but this is by no means the only factor involved in *circus* movements, for *Eristalis* and many other insects perform these movements without tilting. The difference in the rate of movement on opposite sides, owing to tilting, may be a factor in orienta-

tion, as Garrey maintains; but it has in my opinion but little significance for in many insects orientation occurs without tilting and in *Erax* it is, under certain conditions of illumination, actually accomplished by turning toward the side containing the greater extension of the legs, i.e., in the direction opposite that which would obtain if it were due to tilting, in accord with the tonus hypothesis.

If one eye is covered both *Eristalis* and *Erax* turn in non-directive light continuously toward the functional eye. In higher intensities they turn, under certain conditions, more sharply than in lower.

In a horizontal beam of light *Eristalis* with one eye covered deflects strongly toward the functional eye at first, but later it orients fairly accurately. This is probably due to readjustment in the orientation mechanism depending upon experience, but it may be due to a change in the relative sensitiveness of different regions of the retina in the nature of light adaptation, decrease in sensitiveness in the posterior regions being greater than in the anterior regions. This was not observed in *Erax*.

These facts show that orientation is possible in specimens with only one functional eye, and that it is consequently not necessarily dependent upon a balance in the effect of the light on receptors located on opposite sides.

If in *Eristalis* or *Erax* with one eye covered, the stimulus is localized at the posterior edge of the retina, the feet on one side move forward while those on the other move backward, the two front feet deflecting toward the side stimulated, the two hind feet from this side. If it is localized in the lateral portion both front feet move laterally toward the light, as do also the middle feet but to a less extent. If it is localized in the central part of the anterior surface of the eye the feet on both sides move forward and the insect does not turn. If it is localized at the antero-median edge it turns toward the covered eye.

Similar reactions occur in normal specimens if the stimulus is localized in one eye. In general it may be said that stimulation of different regions of the retina in either eye alone sets

up in the legs on both sides coördinated reflexes of such a nature that they tend to direct the organism toward the source of stimulation. Orientation is brought about by a series of reflexes (differential responses to localized stimulation) similar to the scratch reflexes in higher forms induced by stimulation of various points on the surface of the body. In photic orientation the nature of each series of reflexes depends upon the localization of the stimulus in the eye just as the nature of the scratch reflexes depends upon the localization of the stimulus on the surface of the body.

The turning effect of stimulation of a given region of the retina in one eye is obliterated by simultaneous stimulation of the same region in the opposite eye, provided the stimuli are of the same magnitude, and by simultaneous stimulation of any other region in the retina of the opposite eye provided the stimuli in the two eyes bear the proper relation in magnitude. If the stimulus in one eye is located relatively farther forward than that in the other eye, the former in order to produce complete inhibition, must be stronger than the latter, if farther backward it must be weaker.

The elimination of the effect of stimulation in one eye by simultaneous stimulation in the other eye is not due to antagonistic action of the legs on opposite sides as demanded by the Ray or De Candolle theory of orientation as applied to animals by Verworn, Loeb, Bohn, and others. The elimination is due to the total absence of any appreciable effect of the stimulating agent on the muscles of the legs. When an insect is oriented in light, the light has no immediate observable effect on the muscles.

An insect can go fairly directly toward the light without being continuously stimulated and held upon its course because, in the absence of directive stimulation, it tends to take a straight course; and because if it turns slightly from the oriented position the locations of the illuminated regions in the retina change, setting up orienting reflexes which immediately bring it back into the oriented position.

The time-rate of change of luminous intensity in the field undoubtedly plays a part in the orientation of some insects

but orientation is not necessarily dependent upon such changes, at least not in all insects. Orientation may occur without any appreciable changes in luminous intensity in the field or on the surface of the organisms. But even under such conditions, owing to alternate sensitive and refractory periods, the stimulus acts intermittently and the impulses are periodic, there being at room temperature in moderate illumination about 20 per second.

The Bunsen-Roscoe law holds in certain respects for all reactions provided the time factor is sufficiently short; it does not hold for any reactions in any respect if the time factor is sufficiently long; it holds approximately, within certain limits of variation in intensity, in reference to the amount of energy required to initiate reactions (threshold), but it does not hold for any reactions in reference to the relation between the stimulating energy and the magnitude of the reaction.

These facts and others prove conclusively that the tonus hypothesis, or any other that demands balanced action in receptors and locomotor appendages on opposite sides, does not fully account for orientation in insects. They show that orientation in these organisms is dependent upon series of coordinated reflexes in the legs on both sides specifically related to the localization of the stimulus in either eye and inhibition of the effect of illumination in one eye by simultaneous illumination in the other.

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## NOTE ON DOMINANCE IN THE HYBRID PLUTEI OF SEA-URCHINS

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ONE PLATE, SEVEN FIGURES

Several years ago Loeb, King and Moore<sup>1</sup> published an account of the results of crossing reciprocally the two species of California sea-urchin, *Strongylocentrotus franciscanus* and *Strongylocentrotus purpuratus*. The experiments showed that the plutei of each species exhibited strongly marked skeleton characters and that the body shapes of the plutei were also characteristic. Briefly stated, the facts are as follows: Plutei of pure *S. purpuratus* are pyramidal in form, with smooth, straight, club-shaped apical skeleton, cross-bars undeveloped, anal and oral arms short (figs. 2 and 4). The plutei of *S. franciscanus* are larger, since the eggs from which they develop are larger than those of *S. purpuratus*. In shape the pluteus of *S. franciscanus* is rounded at the apex and has long arms; the apical skeleton is curved and covered with spines, the cross-bars overlap or fuse at the center, anal and oral arms are long (fig. 1).

The reciprocal crosses both show the dome-shaped apex, relatively long arms. The skeleton is straight, club-shaped, as in *purpuratus*, but spiny, with cross-bars and with long anal and oral arms like *S. franciscanus* (figs. 5, 6, 7).

Recently the correctness of our descriptions and conclusions has been called in question,<sup>2</sup> so it seems best to publish some photographs of the larvae.

<sup>1</sup> Loeb, J., King, W. O. R., and Moore, A. R., 1910, Ueber Dominanzerscheinungen bei den hybriden Pluteen des Seeigels, *Archiv für Entwicklungsmechanik der Organismen*, Bd. 29, S. 354-362.

<sup>2</sup> Newman, H. H., 1923, Hybrid vigor, hybrid weakness, and the chromosome theory of heredity—An experimental analysis of the physiology of heredity in the reciprocal crosses between two closely associated species of sea-urchins, *Strongylocentrotus purpuratus* and *S. franciscanus*, *Jour. Exp. Zool.*, vol. 37, pp. 169-207.

I take this occasion to thank Dr. T. C. Nelson, of the Zoölogical Laboratory at Rutgers, for making the photomicrographs.

Figure 1 shows the outstanding traits of the pure franciscanus pluteus, figure 2 of the pure purpuratus. Figure 4 is of a parthenogenetic pluteus of *S. purpuratus*, and therefore does not differ essentially from the plutei in figure 2. Figures 3, 5, and 6 are from the results of crossing the egg of *S. franciscanus* with the sperm of *S. purpuratus*. Figures 5 and 6 show the best development. The large size of the plutei proves that they have developed from the large franciscanus eggs, while the club-shaped apical skeletons prove purpuratus paternity: spininess of the skeleton, complete cross-bars (especially clearly shown in figure 5) and long anal and oral arms are inherited from the franciscanus mother. The same characters are to be seen in the two plutei in figure 7. These two plutei show, by their small size that they have developed from purpuratus eggs. The club-shaped apical skeleton further proves purpuratus ancestry, but the spininess of the skeleton, complete development of cross-bars and long anal and oral arms proves franciscanus paternity. The figures therefore contain in themselves sufficient evidence to decide the issue.

Attention should also be called to the fact, which was clearly stated in the paper referred to, that such plutei as shown in figures 5 and 6 could be obtained only in small numbers. Figure 3 represents a defective pluteus of the cross *S. franciscanus*  $\times$  *S. purpuratus*. Since the arms and cross-bars are not developed, the apical skeleton, however, is complete and in its characters give sufficient evidence of the ancestry of the specimen.

The hybridization experiments in question were repeated throughout the three seasons following 1910, with the same results each season; the plutei never showed evidence of the seasonal variation such as that described by Shearer, de Morgan and Fuchs for the forms at Plymouth.

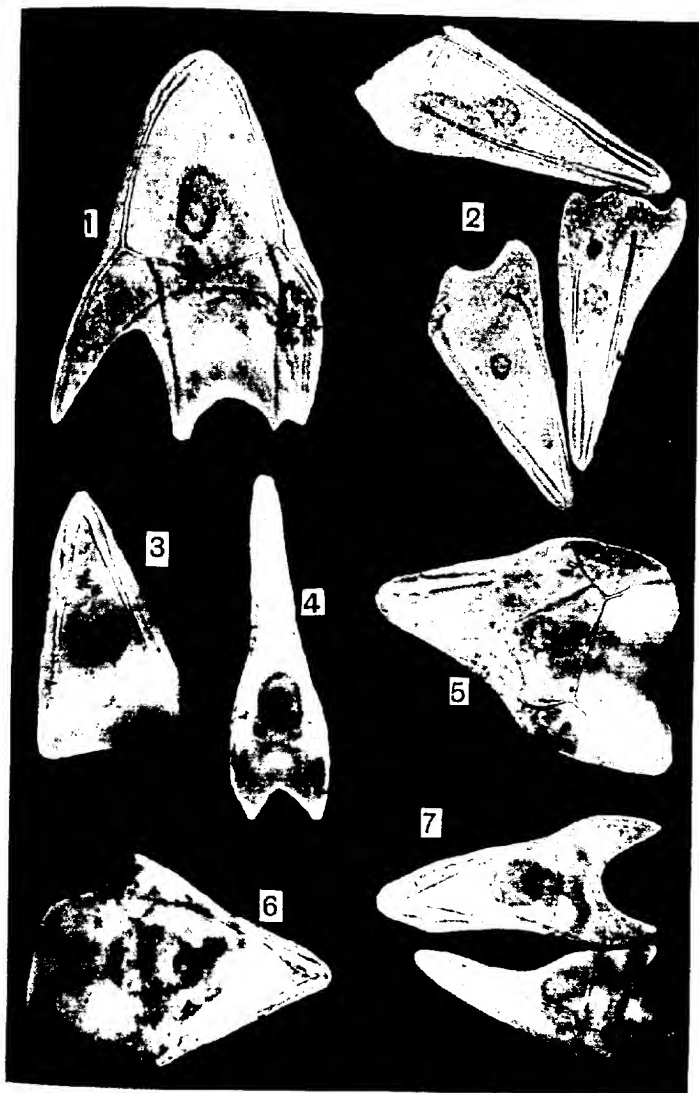
Shearer, C., de Morgan, W., and Fuchs, H. M., On the experimental hybridization of Echinoids. Philosophical Transactions of the Royal Society London, Series B, vol. 204, pp. 255-362.

## PLATE

## PLATE 1

### EXPLANATION OF FIGURES

1. *S. franciscanus* ♂ × *S. franciscanus* ♀.
  2. *S. purpuratus* ♂ × *S. purpuratus* ♀.
  4. *S. purpuratus* ♀ parthenogenetic.
  - 3, 5, 6. *S. franciscanus* ♂ × *S. purpuratus* ♀.
  7. *S. purpuratus* ♂ × *S. franciscanus* ♀.
- The magnification is 190.





## A DEMONSTRATION OF THE STABILITY OF THE GENES OF AN INBRED STOCK OF *DROSOPHILA* *MELANOGASTER* UNDER EXPERIMENTAL CONDI- TIONS

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TWO CHARTS

### INTRODUCTION

Fifty-four years ago Darwin said of the causes of variability, "The subject is an obscure one; but it may be useful to probe our ignorance." While our ignorance of the causes underlying heritable variation is as profound today as it was then, certain developments in biology have so narrowed the field that relatively definite tests are now possible. The problems of variation following hybridization can now be sharply differentiated from those arising in pure breeds, especially in those cases in which the discovery of a gene can be followed by its location in the germinal material. Johannsen's distinction between genotype and phenotype, and the studies on pure lines which have made its general applicability seem probable, also indicate a series of pitfalls which the experimenter is now better prepared to avoid. Thus it is now evident that a mutation may show a range of fluctuation which overlaps that of another heritable character, under the same or different environmental conditions, and still maintain its identity in a cross. It also seems probable that there is no necessary relation between the size and phenotypic conformity of a mutation and its stability in heredity. The origin of the eye colors of *Drosophila* has demonstrated that the sequence in which these mutations arose bears no relation to the color sequence. In other words mutations appear to be transmitted quite independently of somatic variability and to be particulate

in origin as well as in inheritance. Muller ('20) has emphasized this point. Since a mutation usually occurs in one of two homologous loci, "the immediate cause of a mutation is not a diffuse influence existing throughout the body, the cell, or even the nucleus; the mutation is due to an event of such minute proportions . . . that it strikes only a single one of two nearly similar loci in the same nucleus" and "the possibility of influencing the kind of mutation which occurs would seem to recede indefinitely, unless some unique method is found which does not merely consist in an acceleration or intensification of the ordinary processes of mutation." Reference to the pharmacological literature will convince anyone, that in attempting to affect anything of such delicacy we are dealing with an almost unknown field.

It is unfortunate that much of the painstaking labor which has been expended in attempts to throw light on this difficult subject, must be discarded as uncritical because certain necessary precautions have been overlooked. And, indeed, anyone who has labored with a problem of this sort realizes that the vastness of our ignorance on many related topics makes this field of experimentation unusually beset with pitfalls. In fact, each step is in the dark, and all that one can hope is that he has chosen a promising direction. Even then it is difficult to know just how far to follow any one line of investigation which gives negative results. A survey of the literature is by no means encouraging, since it leaves the investigator with a great, and almost inhibiting fear that he is neglecting some simple precaution, which will some day throw his own efforts completely out of court. The most common questions which arise with respect to the experiments in this field are firstly, was the material genetically pure for the character to be tested; secondly, were the developing young treated; thirdly, was starvation and an incidental effect on the vigor of the germ cells responsible for the results obtained; and finally, was selection of small, spontaneously occurring mutations practiced unconsciously during the generations of treatment? Where the effects are general many physiological questions are also of extreme importance. Among these may be cited adequacy of the diet, housing

conditions, and rapidity of mating, all of which have been shown to have definitely adverse effects on the germ plasm (Loeb, '17; Stieve, '18; Papanicolaou and Stockard, '20; Hayes, '18).

The wild type of *Drosophila melanogaster* which was used in these experiments was obtained from the Genetics Division of the University of California. It had been inbred in mass cultures for the preceding six years and was constantly in use in breeding experiments during that time, so that had there been any deviation in its genetic behavior, it would most certainly have been noticed. The wild or plus type of *Drosophila melanogaster* has been described so frequently that it would be both tedious and unnecessary to describe the normal fly. But, in an investigation of this sort, it is of fundamental importance to know the behavior of the stock very intimately, for unless one is able to recognize the amount of fluctuating variability and of mutation which is occurring in the stock, he can obviously not be expected to gauge fairly the variability found in the experiments. For this reason, the progeny of an inbred line (single pair matings) have been kept under close observation during the past two years and a half. Ten pairs each of experimental and control flies were bred for each generation in order to obtain a more nearly complete picture of the condition of the stock as a whole than would be furnished by the progeny of a single pair. As an example of the conditions which make this almost essential, the variation in sex ratio and brood size may be cited. In the controls the brood size averages 206 flies per pair, but this varies for single cultures from 75 to 450 flies per pair. The sex ratios average 98.4 males per 100 females, but varies for single cultures from 56 to 137 males per 100 females. It is obvious that if, by chance, the single culture of the control had an extreme brood size or male value, this fact might make it impossible to gauge the effect of any poison on these two factors. This wide range of fluctuation depends upon the size of the culture bottle, the consistency of the food, death or loss by accident of one or both parents, and, most important and difficult to control of all, varying degrees of infection of the culture medium.<sup>1</sup>

<sup>1</sup> The data for the controls were given in detail in Genetics 8:27-36 (Mann, 1923).

It may be noted at this point that Warren ('18) found the normal sex ratio for three strains of *D. melanogaster* to be 100 females to 95 males and agreed with Moenkhaus ('11) that an average sex ratio is strongly transmissible. Moenkhaus further found that inbreeding, starvation and differences in the food do not change this average.

Since an experiment of this sort necessitates the closest inbreeding, it is of especial interest to note that Castle and his co-workers ('06), Moenkhaus ('11), and Lutz ('11) found that inbreeding has no deleterious effects upon either fertility or vigor if the more fecund are selected to continue the line. The ten culture method makes this possible, since pairs can be selected on the basis of performance. The first group of authors also showed that a fluctuating character, namely, number of teeth on the sex comb, shows no influence as a result of inbreeding. This is entirely in accord with the observations on fluctuations which are recorded below.

The thoracic character 'with' (Morgan and Bridges, '19, fig. 1) was in the stock from the first. It varies with age, the young flies lacking it completely, while all of the mature flies show it to some extent. It also varies with abundance of nourishment, small flies lacking it almost completely. One or more of the large thoracic bristles frequently has a mate, and in a very few cases the same bristle was found doubled on both sides. Since from several matings of two such individuals only one affected offspring appeared, it was evidently simply a fluctuation. Several individuals had one branched thoracic bristle but this is also a fluctuation. It is not uncommon to find flies with more than three lateral thoracic bristles on one side. This consists of the appearance of extra bristles of the size of the small central bristle and seems to be a fluctuation. Single flies are often found with a malformed thorax or abdomen, but these give normal  $F_1$  and  $F_2$  generations when tested. One also frequently finds one or more flies in a generation with one wing longer than the other. They always fail to transmit the character when bred. Some of them are certainly gynandromorphic since a sex comb is found on the fore leg which bears the short wing, while the external genitalia, size, and other characters are female.

The experimental flies were subjected to exactly the same cultural conditions as were the controls except for the particular condition of the experiment. The poisons were weighed and placed in flasks, and banana agar from the general supply was added to the desired amount. The mode of examination was identical in each case.

The problem was suggested by Prof. S. J. Holmes, of the Department of Zoölogy of the University of California. I wish to express my thanks to him for many helpful suggestions in the course of the investigation. Excellent laboratory facilities were provided through the kindness of Prof. E. B. Babcock.

The data on frequency of mutation are still too few to give one adequate criteria for judgment as to effects of environmental conditions, unless the heritable variations occur in rather large numbers in the treated set as compared with the controls. It was accepted at the outset of these experiments that either a general tendency toward abnormality or definite mutating periods must always follow the same treatment of the same inbred stock if the results were to be considered positive.

#### ARSENIC

Arsenic was used in these experiments for several reasons. First, its common use as an insecticide suggested that sub-lethal doses might account for the occurrence of some mutations under non-experimental conditions. Secondly, the fact that arsenic acid is quite harmless, while arsenious acid is only slightly poisonous to yeast (Bokorny, '12), while the latter is decidedly poisonous to animal tissues is of great advantage since no starvation and little tendency toward contamination will attend its use. It has been stated (Davenport, '08) that sub-lethal doses of arsenic inhibit development while (Cushny, '15) very small doses increase growth. The latter suggests that it may be that constant small doses, under good general nutritional conditions, result beneficially, as in the case of the habitual arsenic eaters of the Tyrol, while harmful results follow when arsenic eating is attended by an inadequate diet as in the case of the miners of Reichenstein.

Finally, Cushny states that As is absorbed into the blood, but rapidly disappears therefrom, because it forms a firm combination with the nucleins. O. Hertwig ('13) obtained negative results in an attempt to modify *Rana fusca* and *R. esculenta* sperm with 1 per cent atoxyl. When this was injected into a male frog, sperm taken from the dying male fertilized untreated eggs and gave normal progeny.

When 0.002 per cent of arsenic was mixed with the usual banana agar it was found to check brood size and to produce a certain number of abnormal flies in each generation. 0.001 per cent was used in the main As experiments because the As line tended to die out when 0.002 per cent was used. The abnormality, mentioned above, consists of a general stunting of growth, and especially, in the least extreme cases almost exclusively, in checking wing expansion. With this goes a great increase in the intensity of the dark pigmentation, which shows itself chiefly as an intensification of the "with" markings on the thorax. The thoracic and wing characters greatly resemble those shown in plate 11, figures 7 and 8, of Morgan and Bridges' summary of sex-linked inheritance (Carnegie Inst. Pub. 237). It may, however, be much more intense than that shown in either figure. The abnormality is entirely somatic since it has never reappeared in the progeny of modified flies grown on untreated food, even when care was taken to breed together flies with similar degrees of modification. Four successive generations were bred from such matings but no mutations were found. Somatic notch appeared in these lines during the same period that it occurred in the controls. Matings of abnormal flies are frequently unsuccessful, and such flies are somewhat less viable than the untreated stock. Cultures which contain a large number of modified flies also show a tendency for the pupae to die during metamorphosis or toward an induced weakness which prevents emergence from the pupal case. This does not occur in the control, and it is consequently of some interest to note that in the  $F_1$  from treated parents (grown on untreated food) a number of abortive pupae often occur. This indicates that the same conditions which induce

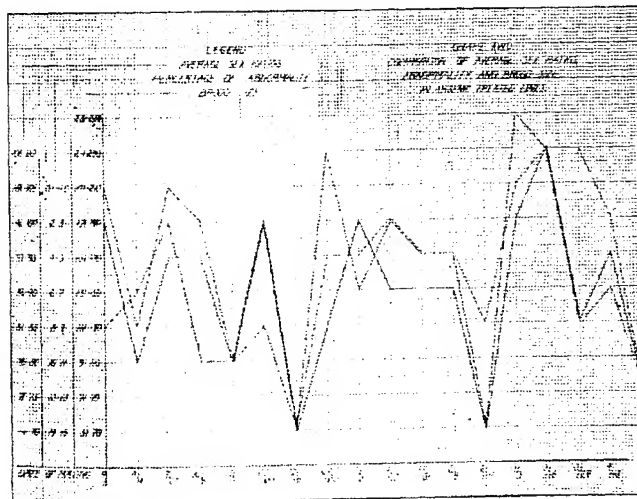
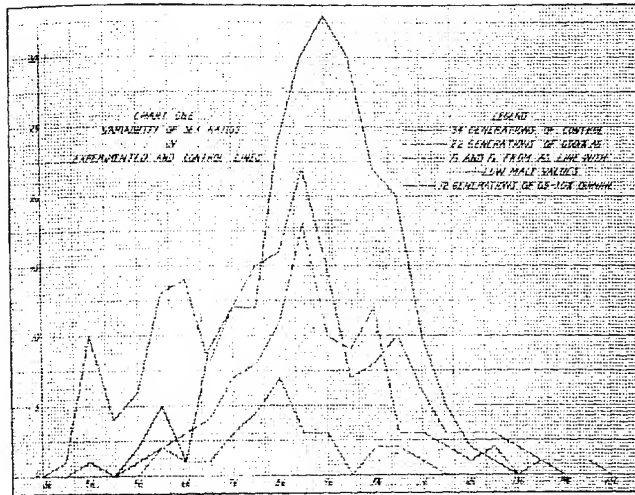
weakness in the parents also affect some of the germ cells in the same general way. Table 1 shows the percentage of abnormalities which appeared in successive generations of As treated flies. It does not increase steadily but shows several sudden periods of increase which seem to be significant since they correspond, in a striking manner, with the low points in average brood size and the low points in the male value of the sex ratio. These facts are shown in chart 2. The average brood size of the treated flies is 137 as compared with 206 for the controls during the same period. The average sex ratio for the treated lines is low, 100 ♀'s to 89.6 ♂'s, compared with the average for the controls for the same period which was 100 ♀'s to 98.3 ♂'s. There has been no selection of lines showing low ♂ value, in fact, quite the reverse is true.

The curve for the arsenic treated line shows (chart 1) a decided skew in the direction of the low ♂ values when all matings are considered separately. Twenty-one matings, during the twenty-two generations of treatment gave ♂ values of 60 or below, while only two control cultures produced ♂ values of 56-60 during 34 generations. Two out of the twenty-one lines giving lethal ratios, when inbred, produced some lethal ratios in their progenies. The sex ratios of these lines are shown below.

GENERATION	SEX RATIO			
	Line from generation 7		Line from generation 9	
	Female	Male	Female	Male
Parents.....	100	57	100	47
F <sub>1</sub> .....	100	50	100	41
F <sub>2</sub> .....	100	66	100	96, 97, 44, 56, 46
F <sub>3</sub> .....	100	108, 97, 44, 50, 56		

While these data indicate that two true lethals appeared in the As line, and none in the controls, they do not necessarily prove that the As caused them. Although it seemed probable that most of the low sex ratios found in the treated stock were simply the extreme of a fluctuation, the As treated line was bred to Xple stock and then back-crossed to Xple, so that should a lethal

appear, it could be detected at once by the absence of plus males from the cultures showing lethal ratios. Four lethal ratios occurred during this portion of the experiment, and most of the male values were very low, but since plus males were found in each brood, and since the reduction of males was evenly distributed to all classes it was concluded that the low male value was due to selection. This is borne out by the fact that there is no sharp line of cleavage between the normal and the lethal ratios, as shown in table 1 as well as by the fact mentioned above that the curves for high percentage of abnormality, low brood size, and low sex ratios correspond so closely. This periodicity seems to show that there are points of low and points of high resistance to arsenic which occur at fairly regular intervals in the history of a line subjected to the same conditions, instead of a gradual acclimatization to it. Since a rigid selection intervenes, it may not seem surprising that following a very adverse period there is an almost immediate rebound to the maximum brood size,  $\sigma^7$  sex ratio value and normality. In any case it would seem that while subjection to the conditions of the experiment is harmful for the time being, selection is at work to counteract these evil effects. In so far as the line is concerned, it seems to have been little harmed by the arsenic treatment during 22 generations. While it is obviously impossible to make any very close comparison between heredity in the fly and in other organisms, these data are still of some interest in that they show that arsenic stunts growth, reduces the number of males and greatly reduces brood size. It also seems to be true that arsenic can weaken the germ cells, at least of modified flies, so that their progeny are weakened even when grown under control conditions, and that, at least in certain cases, the lowered value of the sex ratio persists in some of the progeny. The data are also of interest since they explain why, in the preliminary tests which were made to determine what strength of arsenic to use, widely different results were obtained with the same percentage. It was not until several generations were grown on As 0.002 per cent that it was discovered that a line will not persist upon it, and the line on 0.001 per cent seemed at times almost unaffected by the treatment.



## ALCOHOL

*Literature.* Alcohol has been more used in experiments to test its effect upon subsequent generations than any other drug. The literature on treatment of isolated germ cells seems to show that mature sperm are relatively resistant to alcohol (Ivanow, '13), while ova are most susceptible just before or just after fertilization (Baldwin, '20). The experiments in which an attempt has been made to affect the germ cells by treating the parent, have given very various results. The earlier work was done without adequate controls, with very few animals, and often involved treatment of the female during pregnancy. Only such data as are free from similar objections are included in this account.

Several observers have noted that human alcoholics show in autopsies a tendency to atrophy of the testis, (Bertholet, '09; Weichselbaum and Kyrle, '12; Weller, '21). Arlitt and Wells ('17) showed that of fifteen male rats to which alcohol was fed, only one had normal testes at the end of the treatment. Todde ('10) observed for fowls, and Cole and Davis ('14) for rabbits that breeding effectiveness showed a great reduction after alcohol treatment. The recent work of Loeb ('17), and of Papanicolaou and Stockard ('20), which demonstrates a direct relation between underfeeding and degeneration of ovarian ova, and that of Hayes ('18) which shows that degeneration of elements of the testis follows excessive sexual activity, make one wish for more data on these points with reference to the results cited above.

Alcohol treatment of the parents caused reduced brood size in the experiments of Ceni ('04), and Pearl ('17) with fowls, of MacDowell and Vicari ('17), and Stockard and Papanicolaou ('18) with rodents. Nice ('12, '17) reports increased brood size for mice, while Harrison ('19) obtained results showing generally increased vigor in the progeny of *Selenia bilimaria* following intensive selection of eggs and developing young by alcohol treatment.

A general increase in vigor of the test animals was reported by Pearl ('17), by Nice ('12, '17) and by Harrison ('19). The reverse was shown by the work of Ceni ('04) and Stockard and Papanicolaou ('18).

The male value of the sex ratio was more variable in the progeny of alcoholized fowls (Pearl, '17), and was decreased in that of similarly treated guinea-pigs (Stockard and Papanicolaou, '18).

TABLE 1  
*Summary of data for arsenic experiment*

GENERATION	DATE	TOTAL NUMBER OF FLIES			ABNORMAL FLIES			PER CENT ABNORMAL	AVERAGE SEX RATIO		NUMBER OF CULTURES	NUMBER OF FLOUNDER CULTURE
		Female	Male	SUM	Female	Male	SUM		Female	Male		
1	8 '19 20											
2	9 '1 20	603	582	1,185	12	6	18	1.5	100	96	10	118.5
3	9 '14 20	593	466	1,059	67	32	99	9.3	100	79	8	132
4	9 '27 20	908	871	1,779	15	19	34	1.0	100	95	9	179
5	10 '6 20	337	323	660	9	11	20	3.0	100	95	7	94
6	11 '1 20	505	402	907	69	38	107	14.7	100	79.1	10	91
7	11 '20 20	634	627	1,261	20	24	44	3.4	100	98.9	11	114
8	12 '6 20	166	113	279	26	16	42	14.0	100	68	4	69
9	12 '20 20	625	507	1,132	33	24	67	5.4	100	81	5	226
10	1 '3 21	262	257	519	19	8	27	5.1	100	98	4	132
11	1 '17 21	449	442	891	21	8	29	3.0	100	89	5	187
12	1 '31 21	722	640	1,362	29	34	63	4.6	100	89	9	151
13	2 '8 21	836	747	1,583	38	36	74	4.6	100	87	10	160
14	2 '19 21	331	227	558	31	17	48	8.6	100	68	8	69
15	3 '3 21	947	916	1,863	0	2	2	0.1	100	96	8	232
16	3 '16 21											
17	3 '29 21	668	724	1,392	0	3	3	0.2	100	108	7	198
18	4 '13 21	616	499	1,115	3	0	3	0.2	100	81	9	124
19	4 '26 21	448	429	877	25	7	32	3.6	100	95	6	146
20	5 '15 21	460	330	790	64	45	109	13.8	100	71	10	79
21	5 '27 21	571	552	1,123	43	28	71	6.3	100	96	8	140
22	6 '14 21	442	428	870	6	8	14	1.6	100	96	4	217
23	6 '28 21	465	321	786	11	5	16	2.0	100	69	8	98
Totals, . . . . .		11,608	10,403	22,011					100	89.6	160	137

<sup>1</sup> 0.002 per cent as instead of .001 per cent.

In the same period the average brood for the controls was 192, the average sex ratio 100 females to 98.3 males.

General abnormality was shown to be greatly increased in the progeny of alcoholized guinea-pigs (Stockard and Papanicolaou, '18) while brachydaetyly was increased from 39 to 48.2 per cent

in a backcross of an  $F_1$  hybrid with a normal fowl (Danforth, '19). MacDowell and Vicari ('21) have recently reported results with rats which seem to show that intelligence is slightly lessened in the  $F_2$  from treated parents.

*Experiments.* Since *Drosophila* lives normally in a fermenting medium it has undoubtedly become adapted to small quantities of alcohol. The flies were subjected to alcohol fumes, since miring would undoubtedly result if alcohol enough to produce intoxication were mixed with the food. They reacted to this treatment first, by greatly increased activity; second, by a tendency to fall suddenly to the bottom of the vial, and finally by loss of the power of equilibrium, inability to stand, and complete stupor. It is comparatively easy to overestimate the amount of fumes which the flies will stand, and especially on the first day, severe treatment often results in death. Since selection of parents is undesirable, great care had to be exercised in giving the treatment, the flies being removed to a fresh vial when they became quiescent. The males and females respond to the treatment somewhat differently, and since it seemed desirable that all should have the maximum treatment, the sexes were treated separately. The treatment was begun when the young adults were still hardening.

The first of the alcohol experiments was an attempt to test the efficacy of alcohol as a selective agent. A white-eyed, black-bodied male was crossed with a plus female and twenty males and females were selected from the  $F_1$ . Ten of each were alcoholized one to three times daily for a three-day period, the number of treatments depending upon the rapidity with which they recovered. The males and females of the controls were kept separated during this period so that any differences arising from delayed breeding might be obviated. On the evening of the third day both lots were bred, single pair matings, and allowed to lay eggs for four days. Treatment (males and females separately) was then resumed for a three-day period, followed by remating and breeding as before. Eggs laid during the treatment were saved and the records of the progeny derived therefrom were kept separate.

The first result of the treatment was to increase the brood size of the alcoholized set of cultures. While ten pairs of untreated

parents were producing 2,842 offsprings, ten pairs of alcoholized flies produced 3,236. This is the more remarkable since five males died during the second period of treatment and thus five pairs were eliminated from the second breeding period. This increase in brood size is not a result of selection of parents since the experiment was planned to eliminate this factor. The data for first brood, intermediate period and second brood are given below.

	CONTROL	ALCOHOL
First brood.....	1385	2231
Second period of treatment.....	212	327
Second brood.....	1245	678
Totals.....	2842	3236

Since the treated flies were significantly more prolific than their untreated brothers and sisters, it must be concluded either that alcohol fumes stimulate sexual activity, or that it is beneficial to the germ cells. When the sex ratios are considered it appears that the ♂ value for the treated set is up to the average of the plus stock, while the control set shows a considerable deficiency of males. The value for the alcohol set is 98.5 ♂'s per 100 ♀'s while the control has only 90.8 ♂'s per 100 ♀'s. This also means that general conditions are better in the treated than in the control cultures.

The data for the distribution in the four classes of progeny is given below.

*F<sub>2</sub> results of alcohol experiment 1*

P		GRAY RED		BLACK RED		GRAY WHITE, BLACK WHITE		N:
		Female	Male	Female	Male	Male	Male	
0.001728	Control	1176	514	313	150	514	175	15.13
		1690		463				
0.001776	Alcohol	1263	621	367	215	539	231	15.062
		1884		582				

Harris' method of determining the goodness of fit was used to obtain the values for  $X^2$  and P. This rather wide deviation appears to be due to different causes in the two cases since most of the deviation in the controls occurs in the BR class, while most of the deviation in the treated sets is attributable to the GW class. It is the general experience of workers with *Drosophila* that when conditions are generally most favorable the classes most nearly approach expectation, and that under such circumstances there is no difficulty in classifying the blacks. This might account, to some extent for the fact that the black classes are normal in the alcohol treated group, and not so in the controls. However, the white sets show little deviation in the controls, considerable in the alcohol set. In this case the deviation is due to a lack of GW flies, the BW set being larger than the expectation. Since white is normally highly viable, while black is less so, it is obvious that alcohol does not act selectively on the germ cells in proportion to the relative viability of the adults. On the contrary it looks as if alcohol treatment favored the black carrying gametes, and at the same time discriminated against the white set. In an attempt to analyze these data somewhat more fully the white classes were brought up to expectation, using the actual GR class as the standard in both sets of data. The same treatment was applied to the black classes in the control set. The results seem to corroborate the above conclusion. The data obtained are given below.

	CONTROL						ALCOHOL					
	Data fixed for white classes			Data fixed for black classes			Data fixed for white classes			Actual deviation		
GR.....	+52	1.589	+37	0.828	+91	5.21	+48	1.254	+64	2.251		
BR.....	-83	12.661	-18	0.588	-70	9.19	-30	1.470	-25	1.030		
GW.....	+16	0.468	-37	2.484	-19	0.68	28	1.251	-68	7.618		
BW.....	+9	0.445	+18	1.761	-2	0.02	+12	0.706	+29	4.163		
Total.....		15.113		5.561		15.13		4.681		15.062		

This table shows that when the white classes of the controls are 'fixed,' the deviation remains as before, but that when the black

classes are brought up to expectation the data are well within the probable error. On the other hand when the alcohol set is 'fixed' for white it is even nearer the probability. A striking correlation seems to exist between the differences between observed and calculated ratios in the actual deviation of the alcohol set as compared with the control set in which the black classes have been fixed. What this means, if anything, is not understood.

No abnormal flies appeared in the course of this experiment.

The second experiment with ethyl alcohol was planned to test the effect of this drug on an inbred line. The parents were given the maximum dosage for the first seven days of their lives. They were then bred (single pair matings) and their progenies were examined for abnormality or mutation. The results were entirely negative, the strain remaining quite comparable to the controls for ten successive generations of treatment. The only difference lies in the fact that the somatic character corresponding to  $N_2$  appeared somewhat later in the alcohol treated line. No mutations occurred during the period of observation. The somatic abnormalities were of the same types which have already been described for the controls. In all 7,292 flies from alcoholized parents were examined. The brood size averaged 177 flies per culture as compared with 188 for the controls during the same period. The sex ratio was 100 females to 97.3 males, while that of the controls was 100 females to 98.4 males. It must be concluded that extreme alcoholization for one week of the life of *Drosophila melanogaster* in each generation is insufficient to produce any effect upon the line during the generations of treatment. The parents of generations nine and ten were alcoholized daily throughout their lives but even this extreme treatment did not materially affect either the number or the character of their offspring.

#### METHYL ALCOHOL

The fumes of methyl alcohol are extremely difficult to use, since death so frequently follows what appears to be a comparatively slight degree of intoxication. The first generation, following treatment of the parents for one week, showed no signifi-

cant effect. The broods averaged large and only three notched flies appeared in a total of 1,275 progeny. Normal appearing flies were selected for treatment, but one of these must have been a masked heterozygote of  $N_2$  since 9.5 per cent of his progeny were notched. No other anomalies occurred in the second generation. In the third the brood sizes were very small, having a total of only 514 flies from six parents, an average of 86 flies per pair compared with 206 for the control. Two gynandromorphs were found, but no mutations.

#### QUININE

*Literature.* It is claimed that quinine causes cell lipoids to swell, and, eventually, disintegration of the chromatin. (Moldovan, '13), and De Sandro ('11) noted that quinine feeding causes a decrease in the mitoses of marrow cells. According to Riddle and Anderson ('18) and others, it functions as a protein-sparer. Treatment of sea-urchin sperm in quinine solutions did not affect their fertilizing power, but short exposure of eggs to a smaller per cent was followed by abnormal cleavages. (O. and R. Hertwig, '87). Since it has been shown to check mitoses, to injure the chromatin, to spare protein and finally, to permit polyspermy it was thought worth trying out on *Drosophila* from the point of view of causation of germinal changes. It has the advantage of being more harmful to other fungi than it is to yeasts so that mould and bacterial infection are very rare in culture media containing 0.3 to 1 per cent of quinine sulphate.

*Experiments.* *Drosophila* thrives on such mixtures (0.5 to 1 per cent quinine sulphate), which are so bitter as to be unendurable to human taste. In fact, second broods (after a ten day breeding period) are more commonly obtained in the quinine than in the control cultures. The average brood for twelve successive generations grown on the quinine treated food was 197, and the male value of the sex ratio was 94. During the same period the controls averaged 202 flies per culture, with a male value of 96.6. Table 2 summarizes the data on quinine, while chart 1 represents the data on sex ratio, in comparison with that of the control and the arsenic treated experiments. The arsenic and

quinine curves are both bimodal and have a greater range, as contrasted with the single mode and lesser range of the controls.

One  $N_2$  mutant appeared in the quinine treated set, during the same period that notch was being found in the controls. Otherwise no mutations appeared and the flies were normal except for the occasional occurrence of the same somatic types as

TABLE 2  
*Summary of data for quinine experiment*

GENERATION	DATE	TOTAL NUMBER OF FLIES PER CULTURE		SUM	AVERAGE SEX RATIO		NUMBER OF CULTURES	NUMBER OF FLIES PER CULTURE
		Female	Male		Female	Male		
6	11/ 1 '20	1,075	1,021	2,096	100	95	9	232
7	11/20 '20	322	294	616	100	91	5	123
8	12/ 6 '20	834	736	1,570	100	88	7	224
9	12 '20 '20	1,188	1,189	2,377	100	100	9	264
10	1 ' 3 '21	659	678	1,337	100	103	6	223
11	1 '17 '21	958	860	1,818	100	86	7	259
12	1 '31 '21	974	869	1,783	100	95	10	178
13	2 ' 8 '21	1,205	1,034	2,239	100	85	12	186
14	2 '19 '21							
15	3 ' 3 '21	942	937	1,879	100	99	8	235
16	3 '16 '21	513	478	991	100	93	8	124
17	3 '29 '21	830	832	1,662	100	100	11	151
18	4 '13 '21	621	532	1,153	100	85	7	164
Totals.....		10,061	9,460	19,521	100	94	99	197

In the same period the average brood for the controls was 202; the average sex ratio 100 ♀'s to 96.6 ♂'s.

were found in the controls. An interesting physiological relation exists between the condition of the food and adult vitality. If flies are left on the food from which they hatched for more than two days nearly all of them die, apparently from starvation. But if these same flies are transferred to fresh food, containing the same concentration of quinine, they thrive and produce normal broods.

## MORPHINE

O. Hertwig ('90) showed that morphine hydrochloride was relatively ineffective in producing polyspermy in sea-urchin eggs, two to three hours in 0.6 per cent being required to cause great abnormality. Sperm were perfectly normal after an hour in 0.5 per cent.

*Drosophila* is very resistant to morphine hydrochloride, 0.1 to 0.5 per cent in the food interfering little if at all with normal development. No mutations were found in four successive generations on 0.5 per cent, and the brood size and sex ratios were uniformly high. The numerical data are summarized below.

*Summary for four generations on 0.5 per cent morphine hydrochloride*

GENERATION	BROOD SIZE		AVERAGE SEX RATIO	
	Female	Male	Female	Male
1	230	225	100	97
2	208	218	100	104
3	117	125	100	106
4	262	269	100	102
Totals.....	817	837		

## STRYCHNINE

O. Hertwig ('90) also treated sea-urchin eggs with strychnine and found that weak solutions caused polyspermy, while stronger solutions result in abnormal development, even if polyspermy does not occur. When sperm were treated with 0.01 per cent for three hours they could still move and fertilize normal eggs. The same author ('13) tested *Rana fusca* and *R. esculenta* sperm with strychnine, but no abnormality appeared in the resulting progeny. In the same year O. and P. Hertwig corroborated this and showed that no harmful results follow treatment of sea-urchin sperm with strychnine.

In the experiments with *Drosophila*, 2,354 flies, belonging to seventeen families, which have spent their entire lives on 0.4

to 0.5 per cent strychnine sulphate only three were somatically abnormal. Three successive generations on 0.5 per cent showed that strychnine reduces brood size decidedly and increases the variability of the sex ratio.

The difficulty in obtaining morphine and strychnine in large enough quantities made it necessary to discontinue their use. In any case, the results do not seem to justify the continuance of these experiments since such large quantities can be endured with so little effect.

#### METHYLENE BLUE

Methylene blue has been shown to produce abnormalities not only when sperm are treated directly, but also when the stain reaches the sex cells by way of the blood stream. O. Hertwig ('90) treated sea-urchin eggs with methylene blue. He reported that the amount of stain which the eggs take up depends rather upon the length of exposure than upon the strength of the stain used. The stain does not appear in the nucleus, but tends to remain at the bases of ciliated cells during cleavage. The wandering cells are laden with stain. Cleavage rate is directly proportional to the amount of stain in the egg cytoplasm. This is partly, at least, due to polyspermy which occurs more frequently in deeply than in lightly stained eggs. Hertwig's later experiments ('13) on spermatozoa are of far greater importance. He treated frog sperm for from twenty minutes to two and a half hours with a 0.025 per cent solution of methylene blue. When these sperm were permitted to fertilize normal eggs many abnormal larvae resulted. Likewise, when adult males were given 7 cc. of 0.1 per cent solution subcutaneously, their progeny showed abnormalities. Later in the same year O. and P. Hertwig corroborated the first part of this work, showing in addition that when *Rana fusca* sperm are treated as above and then allowed to fertilize untreated *R. esculenta* eggs, development is parthenogenetic and quite normal when the sperm have been subjected to severe (i.e., long-continued) treatment. Other experiments showed that when sea-urchin sperm are treated with 0.02 to 0.1 per cent methylene blue for two hours no harm results; when

treated for more than two hours decided harm appears in the resulting progeny, while if the treatment is continued for 16 to 18 hours all development is quite normal. To explain this fact, together with the large amount of difference in susceptibility of sperm from different individuals, and even of sperm from the same testis, O. Hertwig postulates a defense reaction of the sperm to chemical changes in the environment. It has already been noted that the arsenic treated lines show a periodic susceptibility to the influence of that poison. It seems quite possible that some such wave of susceptibility occurs in such cases as this one which Hertwig describes, for if individuals were selected at random no two would be expected to be in exactly the same phase of this wave, and they would, as a consequence, be expected to differ in their response to the same treatment.

When larvae of *Drosophila* were placed on food containing methylene blue in various percentages the number of larvae hatching was shown to be inversely proportional to the strength used. In the first tests about 75 larvae were placed on each of 0.001, 0.01, 0.1 and 0.2 per cent, respectively. The results are shown in the following table:

	0.001 PERCENT	0.01 PERCENT	0.1 PERCENT	0.2 PERCENT
Number hatching. . . . .	63	63	8	6

Obviously the critical point lies between 0.01 per cent and 0.1 per cent in so far as hatching of larvae is concerned. Ten pairs of adults from 0.01 per cent (all of whom were brothers and sisters) were placed upon 0.05 per cent but they laid no eggs, either in the first week, which was spent on 0.05 per cent or in the second week on control food. Three males from 0.2 per cent were left on this food until their bodies were stained almost black. Their ocelli became deep blue. These males were bred with virgin untreated females but no progeny resulted, the ♂'s being apparently sterile, since control ♀'s very rarely fail to leave offspring. Ten pairs which had been kept on 0.05 per cent for three days were transferred to normal (untreated) food. They also failed

to leave progeny, although the parents lived for two weeks in some cases. One ♀ which had developed on 0.2 per cent was mated to a ♂ from 0.01 per cent on untreated food. Their progeny was normal and forms the starting point of the following experiments. Ten pairs were next placed on each of 0.01 per cent and 0.025 per cent. The latter were wholly unsuccessful although one pair when transferred to control food gave normal second and third broods. Small broods were obtained from six of the 0.01 per cent cultures. The line was continued by breeding ten pairs each of first generation (0.01 per cent) flies on 0.02 and 0.01 per cent, respectively, on  $\frac{1}{4}$ -pint milk bottles. No abnormalities appeared in either progeny, but the former per cent gave very small broods, averaging 30 flies per culture with an average sex ratio of 100 ♀'s to 66 ♂'s while the latter averaged 115 flies per culture with an average sex ratio of 100 ♀'s and 97 ♂'s. The controls for the same period averaged 192 flies per culture with an average sex ratio of 100 ♀'s and 98 ♂'s. This is shown graphically below.

	AVERAGE SIZE	AVERAGE SEX RATIO	
		Female	Male
Controls.....	192	100	98
0.01 per cent.....	115	100	97
0.02 per cent.....	30	100	66

Since it seemed desirable to test whether or not methylene blue gave results similar to those recorded for arsenic, and especially because it seemed probable that the critical point with regard to the main problem of modifiability of the germ plasma lay between these two extremes (0.01 and 0.02 per cent), 0.015 per cent was chosen as the concentration to be used in the next generation. This generation was an almost complete failure, however, only three cultures out of fifteen showing larvae, and only two of these giving brood as large as those previously found to develop on 0.02 per cent.

It should be understood that the percentage of methylene blue at the surface of the food is decidedly variable. At first

the culture medium appears to be fairly uniformly colored but as the culture ages the surface becomes more and more deeply stained until most of the stain may be concentrated there. The freshly laid eggs are not obviously blue.

#### LEAD ACETATE

*Literature.* According to Cushny ('15), lead poisoning probably results from the constant ingestion of small quantities of lead, and also, probably to a lesser extent from bodily contact with substances which contain it. It remains in the tissues for a long time. Lead acetate, which is fairly soluble, precipitates the protein in the food and this precipitate is harmless to the cells. Cushny further states that lead is less harmful to lower organisms than the other heavy metals, but Bokorny ('12) observed that 0.1 per cent is deadly to beer yeasts.

Several facts indicate that lead is harmful to the germ cells of mammals and fowls. Oliver ('11) states that death in early development followed when hen's eggs had been painted with lead nitrate. Cole and Bachuber ('14) showed that the infertility of hen's eggs is nearly doubled when the female alone is treated, but remains fairly normal when the male is the treated parent. Weller ('15) states that no marked reduction of fertility or increased sterility followed similar treatment of guinea pigs. He has also recently summarized a considerable mass of evidence which shows that lead poisoning of either males or females frequently causes abortion. Cole and Bachuber ('14) and Weller ('15) agree that treatment of the male or female parent with lead acetate causes decreased size and lowered vitality in the progeny. The latter also noted that the offspring of treated males improve in vigor after discontinuance of the lead. No significant data on heritable structural abnormality following lead treatment are to be found in the literature. Weller ('17) could observe no histological differences in the spermatogenesis of guinea pigs which show lead effects on the progeny, except in a few cases of sterility. The numbers treated are small and the sterility may be incidental, not induced.

*Observations.* Unfortunately, mechanical difficulties have made the use of lead acetate with *Drosophila* very unsatisfactory. Soluble lead acetate causes the banana protein to coagulate. This mass drops to the bottom of the medium and is hence unavailable either to flies or larvae. It is obvious that if enough lead is used to exceed that taken up by the proteins, the banana proteins are no longer available as food. This would be of slight importance since (Baumberger, '19) the greatest source of protein for larval growth is derived from the yeasts, but for the fact that small quantities of lead are very harmful to yeast growth.

Flies, left on filter-paper soaked in lead solution (in vinegar) starve to death most rapidly on the stronger solutions. If, however, flies are transferred daily to the usual food, they live without apparent harm on strong concentrations. When larvae are placed in similar solutions they crawl out and often pupate when still rather small. Few adults hatch from such pupae but repeated trials provided eight brothers and sisters, which were mated and became the parents of the first generation recorded here. There seemed to be no advantage in reducing the strength of the lead, since although the larvae lived longer in the weaker solutions, they failed to pupate. Such larvae, transferred to normal food all became surrounded with a coagulated area and died.

The progeny of the first, and of all subsequent generations, have been placed upon culture bottles containing the usual food, which were then filled with masses of paper soaked in 2 per cent lead acetate solution. No mutations occurred in five successive generations, nor was somatic abnormality increased. The broods were large and normal, the male value of the sex ratio rather small. In all 3,772 flies were examined. The data are summarized on page 236.

#### LITHIUM CARBONATE

Bokorny ('12) found that 0.5 per cent of lithium carbonate is very poisonous to yeasts. In the experiments with *Drosophila* 0.03 to 0.05 per cent was used. When the controls, which had been begun at the same time were hatching, the 0.05 per cent

lithium cultures contained larvae, while the 0.03 per cent cultures had uncolored pupae. Of the 954 flies examined in six generations, all were quite normal.

#### COPPER SULPHATE

Bokorny ('12) found that  $\text{CuSO}_4$  is harmful to yeasts. In the *Drosophila* experiments 0.1 – 0.15 per cent was mixed with the food, but it was soon noted that the cultures became disproportionately infected as compared with the controls, and that partly at least as a consequence, the brood sizes were very much reduced. The problem of maintaining a line under such circumstances is so great that it seemed advisable to concentrate upon substances which were easier to use. After two generations of such checking flies of the  $\text{CuSO}_4$  line were prolific and gave normal progeny on untreated food.

*Summary of data on lead acetate*

GENERATION	AVERAGE BROOD SIZE	AVERAGE SEX RATIO	
		Female	Male
1	326	100	95
2	325	100	106
3	93	100	100
4	274	100	96
5	237	100	88

#### TEMPERATURE

*Literature.* The earlier results on hereditary changes produced as a result of stimulation of the parent, and incidentally of the germ cells, by means of temperature, need repetition at some future time when the genetics of *Lepidoptera*, *Reptilia*, and *Amphibia* is better known. At the present time no amount of study of the data provided by Standfuss, Fischer, and Kammerer can lead to anything of value except as they may stimulate genetic, physiological, and 'induction' experiments. The purity of the stock used by Tower ('06) has also recently been questioned by Plunkett ('19). However, his results are so outstanding in that discontinuous, recessive, and mendelizing characters were apparently easily induced by combinations of heat with dry and

also with moist conditions during the growth period of the germ cells of *Leptinotarsa*, that it would be highly desirable to have access to a more complete account of the experiments reported in 1906, as well as to the further tests which the author then reported as well under way. It is especially important that these results be checked on stock which has been inbred by brother-sister mating during a series of generations. In the light of the many observations on *Drosophila*, which show that mutations occur almost invariably as a change in one of two homologous loci, it seems astonishing that all of the 'induced' recessives should have occurred as changes in both chromosomes at the same time. Tower has also ('18) reported the induction of a dominant physiological character in *Leptinotarsa decemlineata*, an increased capacity of the tissues to retain water as a preparation for hibernation in conditions more arid than those to which they were adapted. He suggests that fixation of this character requires time, since he says that its constancy was especially great after six generations in the desert.

Some of the results with temperature appear to indicate that a change in the relative size of parts of the body may be induced in the progeny by a temperature change affecting the germ cells. The work of Woltereck ('11) seems to show that head size may be increased in *Daphnia* as a result of an increased temperature and changed cultural conditions continued over a series of generations, and that when sufficiently fixed, this condition may be maintained through numerous subsequent generations under normal conditions. It seems possible that unconscious selection of slight hereditary changes might account for this result, but any experiment which apparently produced a character gradually would be subject to the same interpretation. Sumner ('15) reported that three of four lots of  $F_1$  progeny from mice grown at an unusually high temperature showed longer ears and tails than the controls, characters which young mice acquire if they develop under similar conditions. Przibram ('18), on the contrary, found that in the rats, increased relative tail lengths caused in a similar manner, were not hereditary. Agar ('13) showed that while the  $F_1$  and  $F_2$  progeny of *Simocephalus vetulus* show some decrease in size as a

result of heat-induced dwarfing of the parents, the  $F_3$ , under control conditions, is quite normal.

Plough's ('17, '21) results indicate that crossing-over can be increased in certain regions of the second and third chromosomes of *Drosophila*, during the thin thread stage of synapsis. Loeb and Bancroft ('11) also working with *Drosophila*, obtained 'a number' of recessive black flies in the fifth generation of treatment with high temperatures. One dark fly appeared in the next, but none in the eleven subsequent generations. Morgan ('14) and Krafka ('20) report that high temperature did not induce mutations in *Drosophila*.

In a recent cytological paper Seiler ('20) reports that extreme heat causes the sex.chromosome of *Taleporia tubulosa* Retz to remain in the egg at reduction, thus increasing the number of potential females.

*Experiments.* Single pair matings were made from the controls and kept at 25°C. until pupation was well started. The cultures were then transferred to the 31.5°C. incubator and kept there through the whole hatching period. In this way all stages of ontogeny were exposed to the increased temperature. Under these conditions the flies hatched one and a half days sooner than the controls which had been started at the same time, and the hatching period was several days briefer. This is largely due to the fact that conditions became unfavorable in all of the cultures kept in the warm room, while the controls remained in good condition. It may also be true that the younger larvae are more adversely affected than the older, and consequently fail to keep the culture clean. It was found that it is impossible to keep successive generations at 31.5°C. for a series of generations, and that flies which have been kept at this temperature during ontogeny and the first few days of adult life are usually incapable of producing broods for at least ten days thereafter, but that they may breed with fair success during the next ten day period. It requires a rather vigorous breeding rate to permit successful single pair cultures, and reduction of vitality probably accounts largely for this result.

The male value of the sex ratio is increased considerably by exposure to heat, but it immediately returns to normal when the generation so treated produces its progeny at 25°C. The results with heat are summarized in the following table:

*Summary of data from heat experiments*

EXPERIMENT	GENERATION	TEMPERATURE	TOTAL NUMBER OF PROGENY	AVERAGE BROOD	AVERAGE SEX RATIO	
					Female	Male
I	1	31.5	1,604	169	100	108
	2	31.5	206	206	100	116
	3	31.5	280	140	100	125
	4	25.0	2,845	158	100	96.2
II	1	31.5	759	152	100	120
	2	31.5	44	44	100	131
	3	25.0	1,315	109	100	100
III	1	31.5	759	152	100	120
	2	25.0	525	125	100	100
	3 <sup>1</sup>	31.5	780	130	100	106
	3 <sup>2</sup>	25.0	449	112	100	96.0
	4 <sup>1</sup>	31.5	1,142	125	100	114
	4 <sup>2</sup>	25.0	947	189	100	95.6

Very small, sterile, and weakly pigmented flies of both sexes were frequently found among the flies which were hatched at 31.5°C. In the first generation which was treated, an unusually large number of flies with one short wing were noted. Only one mutation appeared, apricot eye, a form which was originally found by Dr. R. E. Clausen. Two males appeared in the second brood of one culture in the third generation of heat treated flies in experiment one. The character proved to be sex linked. No other mutants were found in two repetitions with the same stock.

#### COLD

Cultures containing young pupae were also exposed to cold (2.5°C.). No hatching occurred until after return to 25°C. The older larvae die without pupating but the younger larvae and the pupae are simply checked by cold. Extremely small broods were obtained, including some flies with especially narrow wings. Two

generations were bred from these flies but proved to be entirely comparable with the controls. No mutations were found.

#### ACCUMULATION OF GASES

Cultures containing pigmented pupae were sealed by corking and then dipping the top of a  $\frac{1}{4}$ -pint milk bottle into melted paraffin. No hatching occurred until after the cork had been replaced by the usual type of cotton stopper. The larvae climbed up to the top of the bottle and some unusually small larvae pupated. After the cotton stopper had been replaced, hatching was resumed and a normal brood was obtained. Two of these were mated in a 2000-cc. Erlenmeyer flask, and these were stopped as before after pupation had occurred. The same check to hatching and tendency of the larvae to crawl up was noted as before. When a cotton stopper replaced the paraffined cork the larvae reentered the food within ten hours. After twenty-four hours they were resealed, and left alternately unsealed and sealed at twelve hour intervals. A large number of the flies which hatched during the sealed intervals showed wing peculiarities. Some had the wings simply spread, others spread and drooping. A few other anomalies were found, one fly notched in one wing, one with both eyes rough and one with one eye lobed. None of these proved to be hereditary, and large and normal  $F_1$  and  $F_2$  broods were obtained from them.

#### RESULTS AND CONCLUSIONS

1. A stable stock of *Drosophila melanogaster* failed to show increased mutability or tendency to abnormality when treated with arsenic, methyl and ethyl alcohol fumes, quinine, morphine, strychnine, copper sulphate, lithium carbonate, lead acetate, and methylene blue. Negative results were also obtained with high and low temperatures, and accumulation of the gases of fermentation of the culture medium. In the controls one mutation occurred in each 15,165 flies, while in the experiments one mutant was found for every 23,333 flies examined.

2. Arsenic was effective in comparatively small percentages, cutting down brood size, lowering the male value of the sex ratio.

and producing a definite type of somatic abnormality. During 21 generations of treatment with 0.001 per cent these three characters showed six parallel, and rather sudden drops, followed almost at once by sudden recovery of the maximum condition permitted by development in arsenic treated food. This is thought to indicate that the effects of the poison cannot accumulate over a long series of generations, but that the extreme selection which occurs during one phase of the treatment permits the line to be carried on by the strongest individuals only, hence the sudden return to maximal conditions following a drop.

3. Alcohol appears to have some selective effect upon the germinal material, but no relation seems to exist between the relative viability of the adults possessing the recessive characters tested and the selective effect of the alcohol upon the germ cells.

4. Quinine slightly decreases the male value of the sex ratio but improves the condition of the culture medium.

5. Copper sulphate, lithium carbonate, and lead acetate cannot be successfully used with banana agar because of their harmful effect on the yeasts.

6. Methylene blue causes temporary sterility in both males and females but the later broods from such parents showed no increased tendency to mutation or abnormality.

7. A temperature of 31.5°C. during pupation raises the male value of the sex ratio to about 100, but the stock reverts at once to the average for the controls when permitted to hatch at 25°C. Apricot eye, a sex-linked character, appeared in two males in one culture, but no other mutants were found. 2.5°C. had no effect on heredity.

8. Flies hatched in sealed cultures showed greatly increased somatic abnormality.

9. These experiments add further evidence to the great bulk already existing, which indicates that induction of heritable variations is by no means an easy task, and render it increasingly desirable that the few experiments indicating apparent induction be repeated under the most rigorous circumstances possible.

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## RESULTS OF EXPERIMENTS IN HYBRIDIZING SUBSPECIES OF PEROMYSCUS

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SEVEN FIGURES

### INTRODUCTION

During the past few years I have published several preliminary statements of the results of crossing subspecies of *Peromyscus*. These have been contained in Bulletin No. 3 of the Scripps Institution for Biological Research (October 19, 1917), the *American Naturalist* (June-July, 1918), and the *Journal of Experimental Zoölogy*, April, 1920. Since the publication of these papers, data have become available from much more extensive additional series of animals. I have likewise completed the large amount of statistical work necessary to reveal the behavior of a considerable number of characters not hitherto dealt with,<sup>1</sup> and have developed the necessary technique for the measurement of one very important additional character, coat color.

The results of the studies thus far completed are presented in the following pages. The most recent, and in some respects most satisfactory series of hybrids which have been reared at this institution have not, however, been dealt with in the present report. I refer to hybrids between the subspecies of another species of *Peromyscus* quite distinct from that employed by me (*P. eremicus eremicus* and *P. eremicus fraterculus*), which have been reared by Mr. R. R. Huestis. These are being used by Mr. Huestis in a genetic study of microscopic hair characters, upon which he is now engaged. After the completion of his work, it

<sup>1</sup> This has been done with the assistance of Mr. R. R. Huestis.

is my expectation to subject the entire series of pelages prepared by him to colorimetric and statistical treatment such as have been employed in the case of my own hybrids of *Peromyscus maniculatus*.

In view of the rather extensive accounts which I have already published of the objects of these studies, the animals under consideration and the methods employed, it will not be necessary to devote much space to these matters in the present paper. The chief new feature of my technique is the analysis of the coat color by the aid of the Hess-Ives Tint Photometer. A brief discussion of the use of this instrument for the purposes at hand is contained in a recent paper (Sumner and Collins, Jour. Exp. Zool., October, 1922).<sup>2</sup>

Before proceeding with a summary of the results of these investigations, I will take occasion to repeat certain statements which I have more than once made relative to the obstacles which are offered by *Peromyscus* as material for genetic research.

Although these mice breed readily and often live to an advanced age under the conditions of captivity, the growth and reproduction of cage-bred animals is quite appreciably affected. On the whole cage-bred individuals are smaller than wild ones of the same age, and there is a tendency toward certain changes in the proportions of the body, notably a relative shortening of the vertebral column, including the tail, and a shortening of the feet. These effects may be altogether lacking, or they may vary in degree from a condition scarcely distinguishable from the wild type to a condition which may be properly referred to as deformity. Such extreme cases are, however, rare.

The net result of these changes is to bring about a mean reduction of size in series of cage-bred mice, and to alter, in a definite direction, the average length, relative as well as absolute, of certain appendages and of certain bones. Such changes, in so far as they concern some of the very differences by which subspecies differ from one another, are of course a rather serious

<sup>2</sup> Owing to the great potential value of this apparatus for genetic and mammalogical studies, it is my expectation to write a more adequate account of it in the near future.

impediment to the work. Indeed a critic who should seek an opportunity to discredit these studies could easily seize upon these oft-repeated admissions of mine as a plausible basis for attacking the validity of any or all of the conclusions which I may have reached.

The relevancy of such possible criticisms has been dealt with in a number of earlier publications, but certain statements may profitably be repeated here.

1. It must be pointed out that the discussions of the subspecific characters of the parent stocks are based upon wild material, most of which has been trapped at an adult or nearly adult stage of life.

2. The mean differences between series of wild and cage-born specimens of the same race are smaller in degree than the mean differences between the several races, or at least between the more widely separated of these, such as have been used in the hybridization experiments. And in any case, the relative values of a given character in these races are much the same, whether we employ wild series or captive series.

3. It is doubtful whether coat color is modified appreciably by captivity. If any differences exist which are due to this cause, they are slight in comparison with the racial ones.<sup>3</sup>

4. Despite these modifications due to captivity, the individual differences among the very characters which are subject to modification may be shown to be largely of the 'genetic' or hereditary type. Thus, as will be pointed out below, the parent-offspring correlations, even as regards such a highly modifiable character as relative tail length, are in no way inferior to those which are based upon less modifiable characters among these animals, and compare favorably in this respect with those based upon various characters which have been studied in mankind. That correlations of this sort are chiefly due to agreement in genetic composition need hardly be argued. No tendency toward agreement between related individuals of two successive

<sup>3</sup> Adequate series of skins, adapted to testing this point quantitatively, are not available at present. Such series would need to be fairly large, as well as strictly comparable in respect to age, season, etc.

generations, in respect to peculiarities in their environmental conditions, is possible here, particularly in those cases where the parent generation grew up in the wild, while the offspring were reared in captivity.

5. Lastly, it will be shown below that whereas the first cage-bred generation of one of the pure races is commonly more variable than the wild stock—at least for some characters—the second cage-bred generation seems to be actually less variable than the first. This is, of course, a highly important circumstance to bear in mind in comparing the variability of the  $F_1$  and  $F_2$  hybrid generations, since the former (in two of the three crosses here considered) represents the first cage-bred generation, while the latter represents the second. With the evidence at hand, the increased variability of the  $F_2$  generation cannot reasonably be attributed to an increase in the amount of abnormality due to captivity, as I was formerly disposed to think likely.<sup>4</sup>

Another serious drawback to the use of *Peromyscus* as material for genetic research is its relatively low degree of fertility, particularly in captivity. At best, it is rarely possible to obtain more than two or three generations per year, and the broods consist, on the average of less than four young. Add to this the fact that cage-born stock commonly contains a high proportion of totally sterile individuals, amounting perhaps to fifty per cent of the whole.

The difficulties thus far discussed may be ranked as practical or technical ones. It is possible that they could be reduced, to some extent, by properly conducted experiments along the lines of food chemistry or pathology. But a quite different sort of difficulty has been alleged in the case of *Peromyscus*—one of a far more fundamental nature. It is frequently intimated that this group, among others, is quite obviously 'unfavorable' for genetic studies, in so far as the factorial differences between any two of these races are too complicated to be unravelled without a prohibitive amount of work. This assertion deserves some attention at this stage of our discussion, since it is the expression of a viewpoint quite different from my own.

<sup>4</sup> Sumner, Scripps Institution Bulletin No. 3; *American Naturalist*, June-July, 1918.

It is widely assumed that all true inheritance consists in the passing along, through successive generations of germ-cells, of various combinations of mendelian unit factors. The entire heritage of any individual is assumed to consist of a vast number of such factors - to be the sum of these and nothing more. Genetic research, according to this viewpoint, has for its object the isolation of the greatest possible number of these unit factors, the determination of the relationship of these factors to one another in the chromosomes, and the effects of various factors, single or combined, upon the totality of characters of the adult organism. Any studies which are not conducted according to the current methods of mendelian analysis are apt to be stigmatized as superficial or uncritical, and their relevancy to true 'genetics' is questioned. The actual laws of heredity, it is contended, are most clearly revealed by the selection of comparatively simple cases. For the present it is better, as a matter of practical expediency, to let the more complicated cases alone.

One who is disposed to be skeptical as to the pan-mendelian view of heredity and evolution may be tempted to rejoin to this argument that it is the selection of these relatively 'simple' cases, and the persistent avoidance of 'complex' or 'unfavorable' material such as *Peromyscus* that makes mendelian inheritance seem so universal. Leaving aside, however, the question whether all inheritance may be reduced, in theory, to the mendelian type, and also whether, at present, it is scientifically or pedagogically expedient to be dogmatic on this point, we still have to answer the question whether it is not profitable, even now, to study and describe some of these cases which do not obviously conform to the mendelian scheme.

To some of us who are quite as much interested in the geographic and climatic occurrence of variations as in their hereditary transmission it has always seemed that certain natural species of rodents afford particularly *favorable* material for the solution of various evolutionary problems. The major problem of organic evolution in general has always been closely bound up with the minor one of the 'origin of species.' Looking at the matter in a preliminary way, it would seem to be more relevant

to the solution of this problem to investigate some natural species which has become differentiated into a number of well-marked geographic races than to select for consideration some domesticated animal or plant which has been broken up by the breeder's art into a variety of artificial races.<sup>5</sup> At the same time, we freely grant that these last are equally 'natural' in the broad sense of the term.

Once more, the contention has been made, and quite truthfully, that none of these wild races are 'pure,' in the sense that this is true of a long inbred strain of domesticated animals or plants. This is evident from the wide range of variability shown by a collection of any one of the sub-species, and by the fact that these variations are, in a considerable measure, found to be heritable. It would doubtless be possible, by selection and inbreeding, to produce numerous strains of these mice, differing in various ways from one another, but fairly constant within themselves. Any ordinary sample of a wild sub-species is without question 'mixed,' in this sense, and consequently is doubtless unsuited for the solution of certain genetic problems. But it would be folly to contend that no genetic studies of any sort could be profitably conducted upon such 'mixed' material.

The main questions which I hope to answer in the ensuing pages are: 1) How do the characters by which the various subspecies differ from one another behave in hybrids of the first and second generation? Does the entire complex of differences, in any instance, act as a single mendelian character, or do the various component differences 'mendelize' independently of one another, or finally, is there no obvious appearance of segregation at all? 2) Is there any tendency for the characters which are associated together in the parent subspecies to be associated in heredity? Otherwise stated, is there any evidence of linkage among these characters? 3) Is there any constant tendency toward an increase of variability in the second hybrid generation, as compared with the first, and if so, are the relations of a sort which lend support to the 'multiple factor' explanation of the

<sup>5</sup> See Osgood, *American Naturalist*, January-February, 1921.

inheritance of quantitative characters? 4) To what extent do individual differences, either among the members of a single 'pure' race, or among hybrids between these races, tend to be transmitted to the offspring? And particularly, do the  $F_1$  hybrids differ from those of the  $F_2$  generation in respect to the degree of correlation which they show with their parents or with their sibs?

I shall now proceed to give a summarized statement of the results of these experiments, this being followed by a more detailed discussion of my method of procedure and of certain of the data.

#### SYNOPSIS OF RESULTS AND GENERAL DISCUSSION

Three main series of hybrids are considered in the present paper.\* My earliest reports upon subspecific crosses in *Peromyscus*, dealt with certain preliminary series, consisting of very limited numbers of individuals, some of which were unsatisfactory in other ways. These series will not be considered here. Their inclusion would needlessly complicate the discussion, and would not affect the conclusions reached.

##### *Carlotta-Calistoga series*

The first of the crosses to be considered is that between mice which were trapped near Carlotta, Humboldt County, California, and ones which were trapped near Calistoga, Napa County. The former are assigned to the subspecies *rubidus*, the latter to *gambeli*. The Carlotta series of mice differ from the Calistoga ones in a number of respects, some of which are obvious to the eye, some being detectable only by careful measurement. All of these differences relate, however, to averages. For every character considered there is some degree of overlapping of the frequency polygon. Table 1 (p. 276) indicates the most important of these differences, so far as they have been subjected to measure-

\* Two of these have been dealt with briefly in a previous paper (*Journal of Experimental Zoology*, 1920), while the summarized results from all three series have been presented in the Proceedings of the National Academy of Sciences, February, 1923, the latter report consisting of a considerable excerpt from the present paper.

ment.<sup>7</sup> Besides these, various other differences have been observed and recorded, most of which cannot be stated in quantitative terms.

The Carlotta mice have, on the average, relatively longer tails, feet, ears, and skulls, than the Calistoga ones, a broader dorsal tail stripe, lighter foot pigmentation and a darker and less highly colored pelage.<sup>8</sup> On the other hand, the two races do not probably differ in the length of the pelvis,<sup>9</sup> in the indices of asymmetry (= left : right ratio) of certain paired bones, and in the spectral position of the 'free' color (red:green ratio). Certain other mean differences, while they appear to be indicated by the figures of the present table, are very doubtfully to be regarded as actual racial differences between these forms. Thus the mean body length of the Carlotta series slightly exceeds that of the Calistoga series. Likewise the femur length is greater by an amount which would be significant were the number of prepared skeletons of these two races greater.

Of the hybrids between these two races, we have measurements from 154 F<sub>1</sub> and 84 F<sub>2</sub> animals. About 60 per cent of the F<sub>1</sub> hybrids were the offspring of Calistoga mothers. The reciprocal crosses resulted, however, in offspring of approximately equal size and fertility. The hybrids of both generations were killed between the age of six and one-half and seven months.

The relations shown by the mean values for these various characters, in the two hybrid generations, are of minor interest for present purposes, and a consideration of them will accordingly be deferred to the more detailed discussion of our data. I need only state here that in some cases the hybrid values lie between those of the two parent races, while in others they may approximate one of the latter, or they may even lie beyond the means

<sup>7</sup> It must be borne in mind that the measurements of these various parts, as presented in the tables, have been reduced to a common standard (p. 275). They represent relative differences, and are in no way dependent upon differences in the absolute size of the animals.

<sup>8</sup> The meanings of the words 'black,' 'white,' and 'color,' as employed in the tables, and the nature of the 'Red:Green ratio' are explained in the next section of this paper (pp. 281, 282).

<sup>9</sup> Despite some apparent differences in pelvis length, it is very questionable whether there are any actual racial differences in this character, among the races used in these experiments.

for either parent race. In none of these cases, however, are the relations such as to be explicable on the supposition that we have to do with dominance in the mendelian sense. In some instances the two hybrid generations agree rather closely in their mean values, in others they may differ considerably. On the whole, the  $F_2$  values, here and elsewhere, tend to be smaller than the  $F_1$ .

Our chief present interest is with the relative variability of the two hybrid generations. By reference to table 1, a detailed comparison may be made between the standard deviations for each of the characters in the two hybrid generations.<sup>10</sup> There are comprised in the table, nineteen pairs of contrasted figures.<sup>11</sup> In twelve of these cases the  $F_2$  generation shows a higher standard deviation, in six cases it shows a lower one, while in one case the deviations are equal. In only five cases is the difference ( $F_2 - F_1$ ) three or more times its probable error, one of these differences being negative.

It is of interest to classify these differences between the standard deviations of the two hybrid generations, according to whether the characters concerned are ones in respect to which the parent races differ from one another. In ten out of nineteen cases, we have to do with characters of this sort. Of these ten differences, five have the positive sign ( $F_2$  larger), four have the negative sign ( $F_2$  smaller), while in one case the difference is 0. The mean of these differences is  $+0.16$ .<sup>12</sup>

<sup>10</sup> As explained below, the standard deviations for certain of these characters have been 'corrected,' so as to reveal the net variability, after eliminating that due to variability in the general size (body length) of the animals.

<sup>11</sup> It is plain that these figures are not all of coordinate rank. The length of the foot and pelvis have been dealt with separately for the two sexes, in as much as the latter differ materially in the mean size of these parts. Likewise, the four 'characters' into which coat color has been analyzed are naturally closely inter-related. (In every case, 'black' + 'white' + 'color' = 100.)

<sup>12</sup> These are the algebraic differences between the standard deviations divided by their own probable errors. This serves to reduce the various differences to a common unit, so that they are comparable with one another. It also furnishes a measure of the probability of any given difference. Coefficients of variability ( $\frac{\sigma}{M} \times 100$ ) have not been employed here, owing to their very great differences in magnitude for the different characters concerned. Had this been done, the differences between the  $F_1$  and  $F_2$  figures could not have been averaged, as has been possible with the present procedure.

Seven of the nineteen pairs of contrasted figures, on the other hand, relate to characters in respect to which the parent races do not appear to differ from one another. In six of these seven cases, the differences between the standard deviations is positive ( $F_2$  larger), while in only one case, the difference is negative ( $F_2$  smaller). The mean difference (computed as above) is +3.06. Of the two doubtful characters (body length and femur), one shows a difference of +1.1, the other a difference of -3.2.

Thus the tendency towards an increase of variability in the  $F_2$  generation of hybrids is, in this cross, much more evident in the case of characters in respect to which the parent races do not visibly differ from one another than in the case of characters in respect to which the latter differ demonstrably. Add to this, the fact that the only difference between the standard deviations which are three or more times their probable errors relate to characters of the former category. Indeed, three of the most significant among these differences (including one which is seven times its probable error) relate to a class of characters (indices of bilateral asymmetry) which have been shown to be strictly non-hereditary.<sup>13</sup>

Histograms have been constructed (figs. 1 and 2) for three characters whose magnitude is little if at all affected by the absolute size of the animals. These may be profitably inspected by the reader at this point. They will not, however, be discussed here.

#### *Eureka-Victorville series*

This represents a considerably wider cross than the preceding. The Eureka mice, like the Carlotta ones, belong to the subspecies *rubidus*, and they agree closely with the latter in most respects. The coat color, however, appears to be somewhat darker in the mice of the former locality. The Victorville animals, on the other hand, are typical representatives of the desert subspecies *sonoriensis*, and differ conspicuously from *rubidus* in various ways. The differences in tail, foot and skull

<sup>13</sup> Sumner and Huestis, *Genetics*, September, 1921.

length are even more pronounced than in the case of the cross previously considered, as are likewise the differences in the width of the tail stripe, depth of foot pigmentation, and the general coat color. The latter is very much paler in the desert mice than in those from the foggy coastal districts of Humboldt County, while the 'free' color is of different spectral position in the two races, being considerably more yellow in *sonoriensis* and more red in *rubidus*. As regards coat color in general, there is no overlapping of the frequency polygons for these races, and it is highly improbable that an adult skin of either one would ever be confused with that of the other. The desert mice likewise have far less pigment in the soles of their feet, these being, in a large proportion of cases, practically pigmentless.

About the same difference in ear length is to be noted here as in the case of the Carlotta and Calistoga races. As in the latter case, it is doubtful whether there is any real difference in respect to mean body length, the length of the pelvis or femur, or the asymmetry of the paired bones.

This series of crosses yielded ninety-seven animals in the  $F_1$  generation and eighty-seven in the  $F_2$ . It is noteworthy that the  $F_1$  offspring of the Victorville (*sonoriensis*) mothers were much larger, on the average, than those from the *rubidus* mothers, and that their fertility was considerably greater. As in the preceding case, the mice of both generations were killed at the age of six and one-half to seven months.

Reference to table 2 (p. 277) shows that in nearly every case where the parent races differ significantly from one another, the mean values for the respective characters are intermediate in both of the hybrid generations. They are not, however, commonly equidistant from the parental values, nor do the hybrid generations always agree very closely with one another. As in the case of the preceding cross, the  $F_2$  values are preponderantly lower than the  $F_1$ .

It seems worth while at this point to mention the very meagre results of back-crossing an earlier series of Eureka-Victorville hybrids to the two parent races. Only thirteen individuals were reared to maturity, five of which represented the (Eureka-

Victorville)  $\times$  Eureka combination, the remaining eight representing the (Eureka-Victorville)  $\times$  Victorville combination. The skins have been subjected to color analysis and the results conform so closely with 'expectation,' that it is of interest to cite them here, despite the small numbers involved. The former lot are uniformly darker than the latter, there being no overlapping of their ranges. Moreover, in respect to the three values ('black,' 'white' and 'color') which are based upon the colorimeter readings, the former series averages almost exactly three-fourths rubidus (Eureka), while the latter series is approximately three-fourths sonoriensis. It is unfortunate that circumstances have prevented the rearing of larger numbers of back-crosses in the case of all of these hybrids.

Passing to the relative variability of the  $F_1$  and  $F_2$  hybrids of the present series, we find that in fourteen of the nineteen pairs of contrasted figures, the standard deviation is larger for the  $F_2$  generation.

Here again, it is important to group these standard deviations according to whether or not they relate to characters in respect to which the parent races differ. It will be found that eleven of the contrasted pairs of figures relate to characters which differ indubitably in these two subspecies, while eight relate to characters which do not appear to show significant differences, one of these, indeed, relating to a character (femur length) in respect to which the parent races agree very closely. For the first class of characters, we have ten positive differences ( $F_2 - F_1$ ) and one negative, for the second we have four positive and four negative.

In other words, for those characters in respect to which the two subspecies differ from one another, the standard deviation of the  $F_2$  generation is larger than that of the  $F_1$  in ten cases out of eleven. For characters in respect to which the two subspecies do not differ, or only doubtfully differ, the  $F_2$  variability is greater in only four cases out of eight. Again, the mean difference between the standard deviations<sup>14</sup> is +1.15 for the former group, -0.09 for the latter. Thus far, the relations are

<sup>14</sup> See foot-note no. 12.

just the reverse of what they were in the cross previously considered, and conform more nearly with what one would expect on the basis of the 'multiple factor' explanation of these sub-specific differences.

It must be pointed out, however, that in only two cases, among this first class of characters (the diagnostic ones), are the differences between the standard deviations as great as three or more times their probable errors, one of these differences being

a negative one. The largest  $\frac{D}{E}$  quotient of all (5.4) relates to body length, a character in respect to which the two races probably do not differ from one another materially, and here again the difference is negative. The next largest quotient (+4.1) relates to the index of asymmetry in femur length, a character which is known not to be even hereditary.

Once more, the reader may, at this point, profitably inspect the histograms (figs. 3 and 4), though these will not be discussed till later.

*Carlotta-Victorville series*

This is in some respects the most satisfactory of the crosses here considered. The hybrids of both generations were reared to an age which admitted of no doubt as to the full maturity of the pelages. Moreover, the  $F_2$  generation comprises greater numbers (125) than in either of the other crosses, though this advantage is somewhat offset by the necessity of having to deal separately with two different sections of the material, owing to differences of age and of treatment.

As in the preceding *sonoriensis-rubidus* cross, the *sonoriensis* mothers bore considerably larger offspring than the *rubidus* mothers, while in the present case the only  $F_1$  animals which proved to be fertile were derived from mothers of the former race. In this connection, it should be stated, however, that *sonoriensis* when bred *inter se* is much more fertile in captivity than *rubidus*. The sterilizing influence is evidently more potent in its effect upon the female than upon the male.

Both of the races here crossed have already been discussed. With one exception, the statements made in comparing the Eureka and Victorville stocks, will hold in the present case. This exception relates to ear length, which appears to be materially less in the Carlotta than the Eureka series. In fact, it is necessary, in the present case, to transfer ear length from the list of characters in respect to which the parent races differ to that of characters in respect to which they do not differ.

As regards the mean values of the various characters in the two hybrid generations of this cross, we find that, in general, they lie between the means for the parent races, though in some cases they lie beyond either of these last. There is, however, as little evidence of mendelian dominance here as in the crosses previously dealt with. With the exception of two characters (body length and tail stripe), it will be seen (table 3) that the mean values for the linear measurements are lower for the  $F_2$  than for the  $F_1$  series. And without exception, they are higher for the 'later broods' than for the 'earlier broods' of the  $F_2$  generation. The significance of these facts will be discussed in the next section.

Passing, once more, to a consideration of the relative variability of the two hybrid generations, it must first be pointed out that separate standard deviations have been computed for the two sections of the  $F_2$  stock, and that, in general the 'later' broods show a lower variability than the 'earlier' ones, remaining, nevertheless, more variable than the  $F_1$  generation. In the present synoptic discussion, it seems preferable, however, to deal with the mean variability of the entire  $F_2$  generation, i.e., with the mean of the 'earlier' and 'later' standard deviations for each character.

Thus proceeding, we find that in seventeen out of nineteen cases the  $F_2$  standard deviation is larger than that for the  $F_1$ . This fact, viewed uncritically, might be accepted at once as strong evidence of a segregation of multiple factors in the second hybrid generation. But here again, we must classify our characters, according to whether or not the parent races differ in these respects. On this basis, the differences between our standard deviations ( $F_2 - F_1$ ) may be grouped as follows:

	+	-
Characters in respect to which parent races differ.....	9	1
Characters in respect to which such a difference is wanting or doubtful.....	8	1

Thus in nine out of ten cases where the parent races differ, there is an increase of variability in the  $F_2$  generation. But likewise, in eight out of nine cases in which the parent races probably or certainly show no difference, the variability of the  $F_2$  generation none the less increases. It is true that the variability of the racially distinctive character, tail stripe, increases very significantly in both of the  $F_2$  lots, the differences ( $F_2-F_1$ ) being 5.5 and 6.4 times their probable errors, respectively. But over against this is the fact that femur length, in respect to which the parent races agree very closely, displays an equally great increase of variability in both of the  $F_2$  lots, the differences being 6.4 and 5.9 times their probable errors respectively.

The histograms (figs. 5 and 6) show well the range of variation of the two hybrid generations in respect to three characters whose magnitude depends but little upon the general size of the animal.<sup>15</sup> For the characters 'tail stripe' and 'black,' the increase in the range of the  $F_2$  generation is obvious, and suggests at once various diagrams which have been offered in illustration of the 'multiple factor' hypothesis. It must be stated here, however, that the most extreme  $F_2$  figure for tail stripe, and probably a few of the others, are based upon stripes which differed from the usual condition in having a dark central axis, bordered by paler lateral regions. The total width was included in the measurements.

For the case of coat color, another way of expressing the relative ranges of the two hybrid generations is instructive. We may classify the skins according to whether they fall within the limits of one or the other of the parent races in respect to all four of the elements distinguished in the color analysis ('black,'

<sup>15</sup> It would not be very instructive to plot out frequency distributions for such characters as foot length and femur length, for example, since these are so strongly correlated with body length. The variability thus portrayed would largely represent variability in general size.

'white,' 'color,' and the 'R:G ratio'), or whether they are intermediate in this respect. Thus proceeding, we find that in the  $F_1$  generation 57.5 per cent fall within the limits of either the Carlotta or the Victorville race, while 42.5 per cent are intermediate between these two races. In the  $F_2$  generation 69.8 per cent of the skins belong to the former class, 30.2 per cent to the latter.<sup>16</sup>

*Résumé of the three hybrid series*

For each of these series there are nineteen pairs of standard deviations, permitting of a comparison between the  $F_1$  and  $F_2$  variability. There are thus fifty-seven differences ( $F_2 - F_1$ ), of which forty-three bear the + sign ( $F_2$  larger), thirteen bear the - sign ( $F_2$  smaller), while in one case the difference is 0. Such a preponderance of positive differences in itself renders it highly probable that the increase of variability in the  $F_2$  generation is not due to chance. The statistical significance of a large majority of these differences taken singly, is not, it is true, at all certain. But in twenty of the forty-three positive cases the difference is more than twice as great as its probable error, while in ten cases it is three or more times its probable error, and in five cases it is from five to seven times its probable error.<sup>17</sup>

Here, however, as in the discussions of the separate crosses, we must classify the characters whose variability we are considering, according to whether the parent races differ in these respects. As before, we shall distinguish, 1) characters in respect to which the parent races differ indubitably from one another and, 2) characters in respect to which the latter do not differ from one another, or in which the difference is of doubtful significance. Thus proceeding, our figures for these three classes may be tabulated as follows:

<sup>16</sup> The results of such a comparison have not been presented for the other two crosses, for reasons which will be explained below. Even in the present (Carlotta-Victorville) series, the significance of these figures is weakened by the fact that we are dealing with only forty skins in the  $F_1$  generation.

<sup>17</sup> It must be added, however, that in the case of eight of the thirteen negative differences ( $F_2$  smaller), the latter are two or more times the probable errors.

	F <sub>2</sub> VARIABILITY GREATER	F <sub>2</sub> VARIABILITY SMALLER	F <sub>1</sub> AND F <sub>2</sub> EQUAL	MEAN DIFFERENCE (MEAN OF $\frac{D}{E}$ QUOTIENTS)
(1)	24	6	1	+1.07
(2)	19	7	0	+1.33

It will be seen that the proportion of positive cases is slightly greater for the first class of characters (77 per cent than for the second (73 per cent). On the other hand, the mean difference is somewhat less for the former class. Thus, considering our three hybrids and these nineteen 'characters' in the aggregate, the increase of variability in the F<sub>2</sub> generation is found to be somewhat less<sup>18</sup> for those characters in respect to which the parent races differ from one another than for those in respect to which no difference between the parent races is probable.

It is plain, however, that certain classes of characters have been unduly weighted in the foregoing computations. Thus the asymmetry of paired bones has been represented by four different sets of figures, and this is likewise true of coat color, while the length of foot and pelvis have been represented twice, owing to sexual differences. Such a procedure is bound to influence the results, particularly since the indices of asymmetry afford some of the most striking examples of increase of variability in the F<sub>2</sub> generation.

I have accordingly made a second set of computations, in which 'asymmetry' has been counted but once in the case of each cross, the mean of the four quotients in the table being employed. Likewise the figures for 'white,' 'black' and 'color' have been averaged,<sup>19</sup> as well as the figures for the two sexes in the case of foot and pelvis.

The result gives one a somewhat different impression of the relative increase of variability as regards the two classes of characters. For characters in respect to which the parent

<sup>18</sup> Or somewhat *less probable*, whichever way we care to interpret these quotients.

<sup>19</sup> The red : green ratio has, however, been counted as a separate 'character,' since this is largely independent of the other three values.

racers differ, we now have an increase of variability in eighteen cases out of twenty-two, the mean increase being 1.06. For characters in respect to which racial differences are absent or doubtful, we have an increase in nine cases out of fourteen, the mean increase being 0.81. As judged by this method of computation, therefore, there is a somewhat greater increase of variability in the case of characters which differ in the forms crossed.

However, it must be insisted that this difference in the mean values (1.06 and 0.81) is non-significant, statistically speaking. The inclusion of femur length among the characters which distinguish the Carlotta and Calistoga races (which I regarded at first as justifiable) would reverse the relative magnitudes of these figures. We should now have 0.87 and 1.12, respectively, as the figures representing the increase of variability in the characters which do and which do not differ in the present races.<sup>21</sup>

Furthermore, it must be observed that the reality of the  $F_2$  increase is most certain in some of those cases in which the parental difference is least probable (e.g., femur length in the Carlotta-Victorville cross). Indeed, of the six cases in which

the  $\frac{D}{E}$  quotient is greater than four, only one relates to a character in respect to which the parent races are known to differ.

Now I fully realize that more than one previous writer has reported cases in which the range of  $F_2$  variability has increased for characters in respect to which the parent races seem to be identical, and I am quite familiar with the hypothesis which has been devised to account for such cases.<sup>22</sup> But even if we granted all the assumptions necessary, and assumed the existence of genetic differences where no somatic differences were apparent, we should reasonably expect that segregation would be distinctly less manifest in such cases, than in cases where the parent races differed 'somatically' in a conspicuous way. There should at least be a considerable correlation between the genotypic and the phenotypic values for the same character.

<sup>21</sup> See also remarks regarding the coefficient of variability (p. 253).

<sup>22</sup> See Sumner and Huestis, *Genetics*, September, 1921, p. 161.

For the same reason, most of the positive evidence which is currently offered for the multiple factor theory seems to me to be quite inconclusive. We are shown interesting tables or graphs, indicating increased variability in respect to some quantitative character in the second hybrid generation, following a varietal or specific cross. But the significance of such a picture would be entirely destroyed were it shown that an equal increase occurred with respect to characters in which the parent races agreed closely with one another. So far as I recall, such characters have never been included in these tables.

At this point in the discussion I must refer again to a class of characters in which the appearance of 'segregation' is most clearly shown, in spite of the fact that the characters in question are demonstrably non-hereditary. I refer to what I have called the 'indices of asymmetry,' i.e., the quotients obtained by dividing the length (or weight) of the left member of a pair of symmetrical bones by the corresponding value for the right member. These indices have been treated in the same way as the various absolute measurements have been treated, and coefficients of parent-offspring correlation have been computed. The results of these computations (based upon far larger series than are dealt with in the present paper) show pretty conclusively that individual differences in the sinistro-dextral ratios are not hereditary.

Nevertheless, it is a striking fact that these non-heritable characters show the same marked tendency toward an increase in the  $F_2$  generation, as compared with the  $F_1$ , as are shown by undoubtedly heritable characters, which differ in the two parent races. Indeed, the highest single  $\frac{D}{E}$  quotient here found (7.1) relates to asymmetry in femur length, while several of the other differences for these characters are of practically certain statistical significance.<sup>22</sup> The histograms (fig. 7) are based upon the

<sup>22</sup> When the results of the three crosses are combined, as we have a right to do for these characters, and the entire  $F_1$  and  $F_2$  generations are treated as single populations, the certainty is established beyond reasonable doubt. (See Sumner and Huestis, *op. cit.*)

sinistro-dextral ratios for the weight of the two halves of the mandible. They illustrate in an unmistakable manner the tendency of the  $F_2$  variability to surpass that of the  $F_1$  generation.

I shall not here repeat the evidence which can be offered to show that this increase of variability actually results from the fact that we are dealing with an  $F_2$  generation of hybrids, and that it is not due to increasing abnormality or to other causes quite irrelevant to the genetic problems here involved. As regards the special case of the indices of asymmetry, this matter was discussed in considerable detail in the recent paper by Sumner and Huestis, already referred to. And as regards some other characters, the question has been discussed briefly in the introductory section of this paper and will be further dealt with in the section which follows (pp. 284-287).

This increase in the degree of asymmetry shown by paired structures in the  $F_2$  generation presents an interesting analogy with certain results obtained by Gates<sup>23</sup> in crossing large-flowered and small-flowered species of *Oenothera*. Not only was the  $F_2$  generation more variable than the  $F_1$  in respect to flower size, but there were conspicuous size differences between the flowers of the same  $F_2$  plant, and even between the petals of a single flower. It would be worth while to ascertain whether repeated structures in general tend to show greater inequalities of size in  $F_2$  than in  $F_1$  hybrids or pure races.

#### *Coefficients of correlation between related individuals*

In table 4 are given the coefficients of parent-offspring correlation (1) between the  $F_1$  mice and their 'pure' race parents, and (2) between the  $F_2$  mice and their  $F_1$  parents.<sup>24</sup> There are likewise given the coefficients of fraternal correlation, both for the  $F_1$  and the  $F_2$  generations. For the purpose at hand, it has been necessary to limit our consideration to characters whose variations are largely independent of those of the general size

<sup>23</sup> *Journal of Genetics*, March, 1923.

<sup>24</sup> For the Eureka-Victorville series the former correlations are not available (see below).

of the body. Relative tail length, tail stripe, and foot pigmentation are here included; also the four values which are based upon the colorimeter determinations of the pelage (black, white, 'color' and the red:green ratio).

The mean of all the parent-offspring coefficients<sup>25</sup> is +0.268. Omitting the figure for the R:G ratio, a character which does not seem to be hereditary,<sup>26</sup> these values range from +0.200 (white) to +0.298 (foot pigmentation). It is of interest that the highest of these values relates to a character which cannot be accurately measured, as are all the other characters discussed in the present paper, but which is rated according to an arbitrary scale of five grades. These high coefficients of correlation make it evident that the classification adopted, even if not based upon exact measurement, has none the less been made with fair precision.

From table 4, we may compute that the mean of the coefficients for the parent- $F_1$  correlations is +0.213, that for the  $F_1$ - $F_2$  correlations being +0.306. This inferiority of the correlation between the  $F_1$  animals and the parent stocks depends, however, almost entirely upon the Carlotta- $F_1$  coefficients. For some reason, the proportion of genetic to non-genetic variability in the small sample of the Carlotta race here employed is unusually low. The mean of the  $F_1$ - $F_2$  coefficients (+0.306)<sup>27</sup> is almost identical with the mean value which I reported some years ago (1918) for parent-offspring correlations in respect to tail length and tail stripe in three of the 'pure' races (+0.300). If any considerable part of the variability in these  $F_2$  hybrids were due to the segregation of mendelian allelomorphs derived from the parent stocks, we should expect a corresponding reduction in the correlations between the  $F_2$  animals and their  $F_1$  parents. That no such reduction is evident here is not, of course, conclusive evidence against the multiple factor hypothesis, but it shows that

<sup>25</sup> For method of computing this mean see below (p. 292).

<sup>26</sup> More correctly stated, individual differences of this character within a race do not appear to be hereditary to an appreciable extent. There are, however, undoubted *racial* differences, which, of course, are inherited.

<sup>27</sup> The mean figure for tail length and tail stripe alone is practically identical with this.

no considerable part of the  $F_2$  variability can be due to the segregation of factors which were present in a heterozygous state in the  $F_1$  generation.

As is commonly the case, the coefficients of fraternal correlation are here higher than those for parent-offspring correlation. The mean of the entire series is +0.427, those for the  $F_1$  generation averaging +0.442, while those for the  $F_2$  average +0.412. The difference between these figures is probably not significant.

It is generally recognized that fraternal correlation results in part from the tendency for sibs to be subjected to similar environmental conditions, particularly during intra-uterine life. Part of the variability thus represented is consequently of the non-hereditary type. There is small reason for believing, however, that the two hybrid generations here considered differ in respect to the incidence of these environmental agencies, so that this factor may be left out of account in comparing the two sets of coefficients. The subject is more complicated than it would first seem, for the magnitude of fraternal correlation may be affected by circumstances of a statistical nature which have no relation to heredity. But, other things equal, we may say that the segregation of mendelian factors would reduce the fraternal correlation within any population. In the present case this does not seem to have been reduced significantly.

#### *Intra-individual correlation*

For the hybrid series here discussed, along with the parent stocks from which these were derived, and a number of other wild races, coefficients have been computed, in order to ascertain the extent to which various measurable characters are correlated within the individual. The characters chosen are all ones in respect to which the parent races differ from one another. Some other correlations, which are not relevant to the present discussion have been previously reported (1920).

Separate coefficients have been computed for each race or subdivision of our stock while, in the case of three pairs of characters, the two sexes have been dealt with separately. The results cannot be given in detail in the present paper. It is suffi-

cient here to state that a real positive correlation is found to exist between tail length and foot length<sup>23</sup> (+0.272), and likewise between the 'black' of the pelage and the width of the tail stripe (+0.289). A low positive correlation (mean coefficient = +0.107) was likewise obtained in the case of 'black' and relative tail length, but the significance is here doubtful, since five of the fifteen separate group coefficients are negative.

Non-significant mean values (positive or negative) were obtained for the following pairs of characters: relative tail length and tail stripe, foot pigmentation and tail stripe, 'black' and body length, 'black' and foot pigmentation, 'black' and the red:green ratio.

It thus appears, as has been pointed out in a previous paper (Jour. Exp. Zool., '20) that some of the most characteristic elements of the subspecific complex show no tendency to vary together within the limits of a given subspecies. To take a concrete example, *rubidus* as a race has a longer tail and a broader tail stripe than *sonoriensis*. But within neither of these races, is there any tendency for those individuals with longer tails to have broader tail stripes, or vice versa.

It might be suggested, however, that the character differences between the races may behave differently genetically from variations in these same characters within any given race. It is conceivable that correlations among the elements of a complex of subspecific characters might manifest themselves much more clearly in the  $F_2$  generation of hybrids, than elsewhere. I have, accordingly, compared the correlation coefficients for these same pairs of characters in the  $F_2$  series with those of the  $F_1$  series, and with the 'pure' races. There is but one case in which there is any appreciable increase in the correlation between two characters in the  $F_2$  generation. This relates to the correlation between 'black' and tail stripe, for which the mean  $F_2$  coefficient is +0.385, as compared with +0.203 in the  $F_1$  generation. The increase here shown depends, however, entirely upon the two *sonoriensis-rubidus* crosses, while for these the significance of the differences is far from certain.

<sup>23</sup> I.e., independently of the correlation which would be bound to occur in the length of any two members in a population of mixed size.

With this possible exception, therefore, we may say that there is no good evidence, for any single pair of characters, either of a significant correlation in the  $F_2$  generation, where this was lacking in the rest of the stock, or of a higher correlation in the  $F_2$  generation, where this was present in the rest of the stock. So far as can be judged from the few characters under consideration, and for the present rather limited series of animals, there is little tendency for the elements of a subspecific complex to segregate together after a cross. Such characters--if their hereditary basis can be reduced to mendelian factors at all--would seem to be dependent not only upon quite different factors, but upon ones which are not appreciably linked. There is, of course, nothing inherently improbable in such a supposition.

*Recapitulation of the data*

To summarize our data at this point, we may say:

1. The mean values for any given character in the hybrid animals usually lie between the parental values, though they are frequently not equidistant from the latter.
2. On the other hand, the hybrid values may agree fairly closely with one or the other of the parental values, or they may lie beyond either of these.
3. The means for the two hybrid generations frequently agree pretty closely with one another, though this is not the rule. There is a preponderant tendency for the  $F_1$  figures to exceed those for the  $F_2$  generation, this being probably an example of the well-known phenomenon of heterosis.
4. No evidence of mendelian dominance has been found in respect to any single character.
5. Considering all of the crosses here comprised, and all of the characters measured (fifty-seven pairs of contrasted figures), we may say that there is an undoubted tendency toward an increase in variability, as we pass from the  $F_1$  to the  $F_2$  generation.
6. This increase is not significantly greater when we compare the variability of characters in respect to which the parent races differ from one another than when we compare the variability of characters in respect to which the parents agree closely, or at least show no probable difference.

7. This extension of variability in the second hybrid generation is exhibited most consistently of all, perhaps, in the case of the sinistro-dextral ratios of paired bones, although these ratios are known not to be hereditary at all.

8. There is strong evidence that this increase of variability is not due to environmental factors resulting in a higher degree of abnormality in the  $F_2$  generation (pp. 284-287 below).

9. Coefficients of parent-offspring correlation, for all crosses and for all characters considered, average somewhat less than +0.3. The mean coefficient expressing the correlation between  $F_1$  parents and their  $F_2$  offspring is +0.306, which is somewhat greater than that expressing the correlation between the parent races and their  $F_1$  offspring, but agrees very closely with some coefficients which were earlier computed for the pure races.

10. The mean fraternal correlation is fairly large in both hybrid generations, being slightly (though not significantly) greater for the  $F_1$  hybrids than for the  $F_2$ .

11. Coefficients which have been computed for intra-individual correlation show that most of the characters which are associated together geographically (i.e., which vary together, as we pass from one subspecies to another) are, none the less, not appreciably correlated with one another in the individuals of any one subspecies. Nor do the subspecific characters which enter a cross together show any clear tendency to segregate together or even to vary together, in the  $F_2$  generation (except in the case of those characters which are already correlated in the parent stocks).

#### *Discussion*

While the results which are discussed in the present paper are by no means offered as an adequate refutation of the theory of 'multiple factors,' as applied to the differences between the subspecies of *Peromyscus*, they certainly depart rather widely, in some respects, from what we should have expected on the basis of that theory. In another paper<sup>23</sup> I have likewise given reasons

<sup>23</sup> *American Naturalist*, May-June, 1923.

for doubting the origin of these subspecific differences through any such process of 'mutation' as has been studied so intensively in the case of *Drosophila* and various other animals and plants. It will probably be felt by many that my contentions, if sustained, would simply return the whole problem to the confusion in which it lay before the days of Mendel. It will doubtless seem that I have rejected the only scientific hypothesis which has been brought forward in explanation of these phenomena, without offering any other in its place.

At the outset, it should be replied that an incorrect hypothesis is not necessarily better than none. But, after all, the repudiation of the 'multiple factor' theory does not necessarily involve abandonment of any attempt at a scientific explanation of the phenomena at hand.

Even though we may hesitate to postulate the existence of a series of special factors conjured up for the sole purpose of bringing our data into conformity with the generalized mendelian scheme of heredity, we may none the less see the direction in which a possible scientific explanation is to be sought. Surely the indisputable phenomena of maturation and fertilization afford a general basis for an understanding of the relative variability of the two hybrid generations, without the need of postulating various entities whose existence is quite beyond the range of proof.

Thus it seems plain that every nucleus of an  $F_1$  hybrid between *sonoriensis* and *rubidus*, let us say, contains a full set of chromosomes derived from each one of the two races. Barring differences due to the somewhat mixed nature of both of the parent races, the  $F_1$  hybrids are alike in their nuclear composition. But the random assortment of chromosomes, during the formation of the germ-cells of this generation, must bring it to pass that the  $F_1$  individuals differ widely from one another in their nuclear composition, even if allowance be made for a large measure of interchange and mutual modification between the parent chromosomes.

Such an explanation is, of course, substantially identical with the neo-mendelian one up to a certain point. But it stops short

of the latter in refusing to regard the entire heritage of an organism as the sum of a great number of independent and unalterable 'factors' or 'genes' of the type revealed by mendelian experiments. It has not been shown, for example, that these subspecific differences segregate with the chromosomes at all. It is not impossible that they undergo a total or partial blending during some phase of the nuclear cycle. The doctrine of the 'individuality of the chromosomes' has already had to undergo serious modification as a result of the phenomena of 'crossing over,' and it is possible that further serious curtailment is in store for it. There are some reasons, too, for believing that the cytoplasm may play an important rôle in the transmission of certain parts of an organism's heritage.

In any case, one who keeps his mind open to such possibilities is not forced to call in question or to ignore results which do not seem in harmony with the more radical formulation of the factorial hypothesis. While, for example, the present writer can offer no explanation of the curious fact that we have a strong appearance of segregation in respect to certain characters which are not hereditary at all, this fact stands in no contradiction to any hypothesis which he had previously adopted relative to the nature of heredity transmission.

But I shall regard it as unfortunate if the value which is set upon the data contained in the present paper is made to depend wholly on their success in solving some of these fundamental problems of genetics. It must be remembered that almost nothing has hitherto been ascertained regarding the behavior of the subspecific characters of mammals or birds in hybridization.<sup>39</sup> Even the obvious and superficial aspects of the case have been thus far largely unknown.

<sup>39</sup> Bonhote (Vigour and Heredity, London, 1915) made two subspecific crosses of the Egyptian rodent *Meriones crassus*, and reports an entire absence of segregation in the F<sub>2</sub> generation. The number of individuals was, however, very small for such a test.

It is impossible here to enter into any adequate discussion of the literature of specific hybrids.<sup>31</sup> One familiar assertion, which was heard more frequently formerly than now, was to the effect that specific characters tended to blend permanently in hybridization, while varietal characters were held to mendelize.<sup>32</sup> In recent years, many have disputed the reality of this distinction. Heribert-Nilsson,<sup>33</sup> for example, who has conducted extensive experiments in hybridizing willows, is led to the conclusion that even widely distinct species of these trees differ by "a surprisingly small number of factors, but that these factors influence all the organs of the individual" (p. 113). In Heribert-Nilsson's experiments, a close approach to one of the parent forms—at least in some of its most conspicuous features—was sometimes obtained from a very limited series of  $F_2$  hybrids.

From the previous discussion it will be plain that no such account would hold true of the subspecific characters of *Peromyscus*. Most of the elements comprised in the total subspecific complex have been found to be independent of one another in inheritance. Furthermore, no single character of the complex behaves in obvious mendelian fashion. If these characters depend upon mendelian unit factors at all, they are, in every case, dependent upon rather large numbers of cumulative ('multiple') factors, which segregate independently of one another.

The present case appears to be more nearly comparable with certain of those described by Phillips<sup>34</sup> for hybrids among birds. From crosses between the mallard and the Australian duck 'very slight segregation' was noted among 15  $F_2$  males,<sup>35</sup> the

<sup>31</sup> Almost nothing has been done in the way of hybridizing subspecies. But after all, subspecies do not differ in any essential way from species. Whether or not two groups of mammals or birds are regarded as species or subspecies depends upon whether the intergrading forms have been preserved. The extremes are often more widely distinct than two 'good' species.

<sup>32</sup> For example, De Vries: *Species and varieties*, Lecture ix.

<sup>33</sup> *Festschrift utgiven av Lunds Universitet*, 1918, vol. 2, no. 8, pp. 1-145.

<sup>34</sup> *Genetics*, vol. 6, July, 1921.

<sup>35</sup> Only the males are considered in these comparisons, since the most conspicuous specific differences of plumage are confined to this sex.

'most extreme mallard type' scarcely differing from the  $F_1$  individuals. In crossing the mallard and pintail ducks (a generic cross), "the smallest possible amount of segregation was found, both in the straight  $F_2$  generation [sixteen males] and in back-crosses with the pintail male." Also in a somewhat more extensive series of crosses between the gold pheasant and Lady Amherst pheasant, "The  $F_2$  generation . . . is merely a repetition of the  $F_1$ , but with the extreme variates slightly extended."

On the other hand, in crosses between the mallard and Florida duck from which thirty-eight  $F_2$  males were obtained, Phillips records the occurrence of two nearly pure mallard types and three nearly pure Florida types. Some other crosses yielded decided, though rather less pronounced, appearances of segregation in respect to various specific color markings of the male plumage.

Phillips, like most recent geneticists, views the cases which show clear-cut evidences of segregation as the typical ones, regarding the condition of the others as due to the complexity of the factorial differences. An alternative interpretation is of course to suppose that we have all gradations between a complete and permanent blending of characters,<sup>36</sup> and a practically complete segregation of these. More evidence is certainly necessary before we are entitled to choose definitely between these two alternatives.

Further evidence of the unwisdom of allowing oneself to be stampeded at the present time is derived from the recent experiments of J. W. H. Harrison upon lepidoptera.<sup>37</sup> This writer who, a few years ago, was an ardent supporter of the 'multiple factor' hypothesis, has more recently been led to believe that unit characters may be changed as a result of crossing. Harrison holds "that in species- and race-hybrids this gametic blending or contamination is more prone to occur than in crosses between varieties of the same species." This view he bases, in

<sup>36</sup> I will not say of *factors*, since this mode of expression would concede the universality of the factorial scheme of heredity, a view which I regard as quite unproved at present.

<sup>37</sup> See, especially, *Journal of Genetics*, vol. 9, Feb., 1920, and vol. 10, July, 1920.

part, upon "the disparity between the enormous fluctuation of the melanism, even to its actual disappearance, in the  $F_2$  *crepuscularia-bistortata* hybrids [a specific cross], and the uniformity of the melanics in the  $F_2$  *crepuscularia-delamerensis* mongrels [a varietal cross]."<sup>38</sup>

The experiments of Detlefsen in crossing the domesticated guinea-pig with a wild species of cavy (*Cavia rufescens*) yielded many results of interest, some of which the author regards as evidence of mendelian segregation.<sup>39</sup> Thus the 'agouti' (banded) character of the hair displayed characteristic differences in the two species used, and Detlefsen believes his evidence to prove that the two types of agouti are allelomorphic to one another, the wild type being recessive. One circumstance, however, may lead one to question whether the difference depends upon stable factors, which cannot be changed by crossing. The 'wild' agouti condition—at least in some lines—became continually reduced by the successive crossing of these hybrids with non-agouti varieties of the guinea-pig. Since no such reduction is known to follow from the continued crossing (and back-crossing) of agouti guinea-pigs with non-agouti ones, may it not be that such a dilution is rendered possible by the fact that, in the former case, the allelomorphs came from distinct species? Is not this perhaps just such a 'contamination' as Harrison has described in the case of specific crosses?<sup>40</sup>

As regards bone measurements, we learn that "there is little, if indeed any, evidence of segregation and recombination of factors for size in these crosses" (p. 71), although the parent species differ considerably in size. Also, "the differences in skull

<sup>38</sup> 1920, p. 82.—It should be noted that the uniformity last referred to is not a uniformity of the  $F_2$  generation as a whole, but a uniformity of the melanic individuals within that generation.

<sup>39</sup> Carnegie Institution publication no. 205, 1914.—Unfortunately the  $F_1$  hybrids were sterile inter se, so that, for later generations, Detlefsen was restricted to continued back-crosses between  $F_1$  animals and guinea-pigs.

<sup>40</sup> I am interested to learn from Dr. Detlefsen that he himself suggested the possible analogy between these two cases in a letter to Dr. Harrison, written some time ago. Dr. Detlefsen remarks in this connection: "I am likewise somewhat skeptical of an absolutely stereotyped mendelian interpretation for all genetic phenomena."

shape between the wild and tame were blended in the  $F_1$  generation. In later generations [of back-crosses with the guinea-pig] all traces of the pointed, wild skull shape were gradually lost" (p. 77).

While many results from specific hybrids could doubtless be cited which afford more convincing evidence of mendelian segregation than the foregoing, we are, I think, forced to the conclusion that any attempt to universalize this principle is at present dogmatic and premature.

#### MORE DETAILED DISCUSSION OF CERTAIN FEATURES OF THE WORK

I have relegated to this section certain details, both of method and results, which are necessary to a proper evaluation of these experiments, but which would have broken the continuity of the narrative if introduced earlier.

Certain explanations are necessary regarding the tables (1 to 3) giving the means and standard deviations for the various characters in both 'pure' and hybrid stocks. For characters which vary to a large extent with the general size of the animal (body length) the mean values in these tables have been made comparable with one another by reducing them to the values which they would have had if every series of animals had a mean body length of 90 millimeters. This, it is hardly necessary to state, has been accomplished by the use of the 'regression coefficient,'  $x = r \frac{\sigma_x}{\sigma_y} y$ , which need not be further explained here.<sup>41</sup> This procedure has been adopted in the case of relative tail length ('tail per cent'),<sup>42</sup> and the length of foot, ear, pelvis, femur and skull. Since the means for body length in the various series do not in any case depart very far from 90 mm. the results here obtained are doubtless accurate enough for present purposes, despite the

<sup>41</sup> See Sumner, Jour. Exp. Zool., April, 1920, p. 385.

<sup>42</sup> The application of this correction to relative tail length is responsible for the not very great discrepancies between the figures to be noted in the present paper and ones which were contained in a former paper (1920) for some of the same animals.

TABLE I

	CALCOTTA				CALISTOGUA				F <sub>1</sub>				F <sub>2</sub>				D E
	Mean	No.	σ	Mean	No.	σ	Mean	No.	σ	Mean	No.	σ	Mean	No.	σ		
Body length.....	99.07±0.31	116	4.89±0.22	88.73±0.21	121	3.98±0.17	87.54±0.19	151	3.52±0.13	86.33±0.28	84	3.79±0.20	84	3.79±0.20	84	+1.1	
Tail (per cent).....	104.12±0.36	108	5.51±0.25	85.18±0.32	118	5.20±0.23	91.73±0.31	152	5.39±0.22	93.28±0.42	84	5.71±0.30	84	5.71±0.30	84	+0.3	
Foot, c <sup>3</sup> .....	21.32±0.05	63	0.61±0.04	20.15±0.05	67	0.57±0.03	21.20±0.05	81	0.56±0.03	21.18±0.06	81	0.55±0.04	81	0.55±0.04	81	-2.2	
Foot, 2.....	21.08±0.07	53	0.75±0.05	19.76±0.06	52	0.60±0.04	21.01±0.05	73	0.64±0.04	20.82±0.07	43	0.64±0.06	43	0.64±0.06	43	0	
Ear.....	17.21±0.05	116	0.73±0.03	16.81±0.05	119	0.76±0.03	17.69±0.05	153	0.88±0.03	17.20±0.06	83	0.76±0.04	83	0.76±0.04	83	-2.4	
Pelvis, c <sup>3</sup> .....	17.97±0.17	19	0.81±0.12	7	7	17.01±0.04	81	0.39±0.03	17.14±0.05	41	0.47±0.04	41	0.47±0.04	41	0.47±0.04	-2.4	
Pelvis, 2.....	17.98±0.06	28	0.48±0.04	17.72±0.09	21	0.59±0.06	17.66±0.05	73	0.63±0.04	17.61±0.07	42	0.60±0.05	42	0.60±0.05	42	+0.5	
Femur.....	15.98±0.08	39	0.72±0.06	15.14±0.05	21	0.36±0.04	15.21±0.05	151	0.87±0.03	15.37±0.05	82	0.71±0.04	82	0.71±0.04	82	-3.2	
Skull.....	25.52±0.05	39	0.43±0.03	24.88±0.05	21	0.33±0.03	25.67±0.02	128	0.63±0.03	25.56±0.04	83	0.51±0.03	83	0.51±0.03	83	+2.8	
Asymmetry, pelvis.....								156	0.81±0.03					86	1.35±0.07	86	+7.1
Asymmetry, femur length.....								156	2.07±0.08					76	1.60±0.09	76	+4.0
Asymmetry, femur weight.....								149	1.25±0.05					83	5.90±0.31	83	+2.9
Asymmetry, jaw weight.....								151	4.81±0.19	37.25±0.44	83	5.90±0.31	83	5.90±0.31	83	+2.9	
Tail shape.....	41.53±0.31	107	5.13±0.21	31.39±0.35	116	5.53±0.21	37.19±0.26	151	4.81±0.19	37.25±0.44	83	5.90±0.31	83	5.90±0.31	83	+2.9	
Foot pigmentation.....	2.47±0.06	94	0.90±0.04	2.91±0.05	120	0.87±0.03	3.60±0.05	151	0.80±0.03	3.10±0.06	84	0.80±0.04	84	0.80±0.04	84	-1.2	
Back.....	86.70±0.14	38	1.31±0.10	83.30±0.23	28	1.77±0.16	86.52±0.13	60	1.45±0.09	87.47±0.11	58	1.40±0.08	58	1.40±0.08	58	+0.3	
White.....	8.04±0.07	38	0.62±0.05	9.39±0.11	28	0.83±0.07	8.22±0.06	60	0.70±0.04	7.66±0.06	58	0.86±0.04	58	0.86±0.04	58	+2.8	
Color.....	5.96±0.12	38	1.06±0.08	7.30±0.16	28	1.25±0.11	5.26±0.10	60	1.15±0.07	4.84±0.07	58	1.00±0.05	58	1.00±0.05	58	-1.7	
R:G.....	3.51±0.06	38	0.53±0.04	3.32±0.07	28	0.58±0.05	3.23±0.04	60	0.41±0.03	3.20±0.05	58	0.72±0.04	58	0.72±0.04	58	+6.2	

TABLE 2

	ECORA				VICTORVILLE				F <sub>1</sub>				F <sub>2</sub>				D E	
	Mean	No.	$\sigma$		Mean	No.	$\sigma$		Mean	No.	$\sigma$		Mean	No.	$\sigma$			
Body length.....	90.25	6	24.136		4.51	0.17			1.18	0.17			5.71	0.28			3.88	0.20
Tail (per cent).....	104.21	0.30	111		5.25	0.21			8.17	0.31			4.41	0.22			5.03	0.29
Foot, $\sigma^7$ .....	21.48	0.01	86		0.53	0.03			19.57	0.04			0.57	0.03			0.69	0.05
Foot, $\tau$ .....	21.06	0.05	59		0.56	0.03			19.56	0.05			0.62	0.04			0.85	0.07
Ear.....	17.62	0.05	110		0.78	0.03			17.26	0.04			0.75	0.04			0.78	0.04
Pelvis, $\sigma^7$ .....	17.12	0.04	80		0.55	0.03			17.33	0.04			0.67	0.04			0.62	0.04
Pelvis, $\tau$ .....	17.65	0.07	53		0.81	0.05			18.03	0.06			0.72	0.05			0.71	0.04
Femur.....	15.88	0.03	135		6.57	0.02			15.94	0.03			0.67	0.03			0.51	0.03
Skull.....	25.60	0.03	134		0.18	0.02			24.90	0.03			0.48	0.02			0.38	0.02
Asymmetry, pelvis.....																		
Asymmetry, femur length.....																		
Asymmetry, jaw weight.....																		
Tail stripe.....	42.26	0.34	119		5.52	0.24			28.12	0.24			1.89	0.24			5.15	0.28
Foot pigmentation.....	2.85	0.13	20		0.85	0.09			0.90	0.07			0.85	0.04			0.95	0.05
Black.....	89.67	0.13	20		0.84	0.09			78.06	0.10			1.80	0.12			2.08	0.10
White.....	6.62	0.08	20		0.52	0.08			11.00	0.12			0.96	0.06			1.13	0.06
Colors.....	3.70	0.10	20		0.64	0.07			10.91	0.18			1.41	0.09			1.15	0.07
R: G.....	3.56	0.08	20		0.33	0.06			2.94	0.03			0.50	0.03			0.53	0.03

TABLE 3

TABLE 3																					
VICTORVILLE																					
CARLOTTA			F <sub>1</sub>			F <sub>2</sub> (EARLIER BROODS)			F <sub>2</sub> (LATER BROODS)			D			E						
Mean	No.	$\sigma$	Mean	No.	$\sigma$	Mean	No.	$\sigma$	Mean	No.	$\sigma$	Mean	No.	$\sigma$	Mean	No.	$\sigma$				
99.07±0.31	116	4.80±0	22.88	99±0.21	110.1	18±0	17.89	45±0.40	96	5.97±0	29.90	63±0.38	59.4	35±0.27	-4.00	1.26±0.36	4.28±0.25	-4.4			
104.42±0.36	108.5	5.34±0	25.81	17±0.31	136.5	35±0	22.94	18±0.33	96	1.73±0	23.81	75±0.51	58.5	63±0.36	+2.18	1.88	10±0.30	65	4.73±0.28	0	
21.52±0.05	63.0	6.40±0	04.19	97±0.03	75.0	56±0	03.21	65±0.06	47	0.61±0	01.20	62±0.10	28.0	73±0.07	+1.79	75	-0.07	32	0.62±0.05	+0.2	
21.08±0.07	53.0	7.51±0	06.19	36±0.05	61.0	57±0	03.21	31±0.07	48	0.77±0	05.20	22±0.10	32.0	83±0.07	-0.57	70	71±0.08	31	0.70±0.06	-0.9	
17.24±0.05	116.0	7.23±0	03.17	26±0.04	138.0	66±0	03.17	81±0.05	96	0.69±0	03.17	21±0.09	59.0	98±0.06	+1.31	76	-0.07	66	0.79±0.05	+1.7	
17.97±0.12	100.81±0	12.17	33±0.04	78.9	53±0	03.17	10±0.05	48	0.53±0	01.16	53±0.08	27.0	59±0.05	-0.91	16	95±0.08	32	0.64±0.05	+1.7		
17.98±0.06	28.0	9.18±0	01.18	03±0.06	62.9	67±0	01.17	36±0.07	48	0.67±0	03.16	81±0.11	31.0	90±0.07	+2.31	11	14±0.09	34	0.76±0.06	+1.2	
15.98±0.08	39.0	7.2±0	06.15	94±0.03	131.9	51±0	02.15	29±0.05	96	0.70±0	03.14	39±0.11	58.1	19±0.07	+6.11	11	05±0.09	60	1.15±0.07	+5.9	
25.52±0.05	39.0	13±0	03.14	90±0.05	139.0	52±0	02.25	72±0.03	96	0.50±0	02.21	41±0.05	57.0	55±0.03	+1.12	51	-0.04	66	0.50±0.06	0	
										71	0.71±0.04			102.0	81±0.04	+1.8					
										97	1.61±0.08			122.1	78±0.08	+1.2					
										98	2.42±0.12			121.2	75±0.12	+1.9					
										93	1.31±0.06			101.1	63±0.08	+3.2					
										95	4.48±0	22.32	12±0.64	57.7	25±0.45	+5.53	21	-0.64	66	1.76±0.46	+6.4
										96	0.93±0	01.2	10±0.08	59.0	90±0.06	-0.4	2	32±0.09	66	1.04±0.06	+1.5
										40	2.18±0	16.82	42±0.29	59.3	28±0.20	+4.23	40	-0.21	67	2.55±0.15	+1.7
										30	0.96±0	07.9	28±0.11	59.1	39±0.08	+3.2	9	15±0.08	67	1.00±0.06	+0.4
										49	1.71±0	13.8	31±0.22	59.2	17±0.15	+3.7	15	-0.16	67	1.99±0.12	+1.4
										40	0.35±0	03.3	17±0.01	59.0	51±0.03	-3.8	3	25±0.03	67	0.42±0.02	+1.9

Both F<sub>2</sub> lots combined

fact that the correlations between these characters and body length are far from being linear. The tail and foot, for example, are relatively larger in the smaller individuals. In the case of the former member, this is evident from the fact that we find a negative correlation ( $-0.15$ ) between body length and relative tail length (ratio to body).

In the case of the characters mentioned, their standard deviations have been similarly 'corrected,' so as to eliminate that part of their variability which is due to the varying size of the organisms. This has been accomplished by multiplying the crude standard deviations by the factor  $\sqrt{1-r^2}$  in which  $r$  is the coefficient of correlation between the part in question and body length. Since two series under consideration may differ widely with respect to their range in size, it does not seem legitimate to compare the gross variability of their single parts.

Coefficients of variability were not computed, for reasons stated in foot-note on page 253. The use of these, instead of standard deviations, would, however, have left the relative variability of the two hybrid generations unaffected in all cases except two. In these two cases, the signs are changed from positive to negative, i.e., the variability, as thus rated, is found to be lower in the  $F_2$  generation. It is of interest that both of these changes relate to characters (foot pigmentation and 'white,' table 3) in respect to which the parent races differ from one another.

The columns headed number ('no.')

 give the number of individuals of each race which were measured for each character. It will be seen that the numbers in a given column vary rather widely, a fact which demands some explanation. Every animal used in these studies (with certain exceptions to be referred to presently) was put through a series of preliminary measurements shortly after death. These included the length of body, tail, foot and ear, and the width of the tail stripe.<sup>12</sup> The numbers representing the measurements of these characters are consequently approximately equal. The lesser number of those for

<sup>12</sup> See my 1918 paper (above cited) for method of taking measurements.

characters other than body length is due to the impossibility, in certain individuals, of obtaining accurate measurements, owing to the injury of one or another part.

Skeletons were not prepared for certain series, except for the limited number of individuals which figured as parents of hybrids. And even in these cases, certain bones, particularly the pelvis, were sometimes so damaged that accurate measurements were out of question.

Foot pigmentation was not selected as a character for quantitative determination until midway in the course of these studies. The feet of the earlier series of Eureka and Victorville mice were consequently not saved, the small numbers represented in the table being obtained later. There is not, however, the slightest question in regard to the wide difference between the feet of the Eureka and Victorville mice, despite the small numbers here listed. Series of freshly killed animals have been compared at various times in respect to this character, in addition to the more accurate gradation of prepared feet, represented in the tables.<sup>41</sup> Furthermore, the Eureka mice may be assumed to resemble the Carlotta ones rather closely in this respect, and of the latter I have a quite adequate series.

It is with regard to the pelage characters that the discrepancies in the numbers are greatest. Owing to the amount of labor involved in the preparation of skins, and to the fact that I did not at first expect to be able to measure the color characters, no large series were prepared for the wild races. In the case of the  $F_1$  hybrids, I unfortunately restricted the skinning to animals which were parents of  $F_2$  broods, or which were the sibs of such parents. This resulted, for example, in the abridgement of the Carlotta-Victorville  $F_1$  series to forty skins, a number inadequate for the study of variability. On the other hand, all of the  $F_2$  animals were skinned.

Another point in reference to my general procedure should be referred to here. In the case of wild mice, individuals under 80

<sup>41</sup> These last were first placed in 70 per cent alcohol, being later transferred to glycerine. Care has been taken throughout to keep them from exposure to light, except when under observation.

mm. in body length have not been measured nor included in my studies. This was in order that the series should not contain any very immature individuals, though it is true that mice of from 80 to 85 mm. are frequently in their juvenile pelage. Unfortunately, as I now believe, this practice was extended to the cage-born mice, resulting in the exclusion of a certain proportion of stunted individuals, which were none the less known to be fully adult. The measurements of the hybrids comprised in the tables (except those for coat color) are consequently restricted to individuals of 80 mm. or over. This procedure has not, however, affected the results to any material extent, since the number of rejected individuals was in every case small. This I have tested by computing new standard deviations for body length for each entire lot, including these much undersized individuals. The relative variability of the hybrid generations, in this respect, is left quite unaffected by this procedure, and it is fairly certain that the standard deviations for the other characters would not be materially affected thereby, either relatively or absolutely.

As just stated, the skins of these undersized (but mature) mice were included in the hybrid series, a circumstance which is responsible for the fact that there are sometimes comprised in the tables a greater number of skins than of mice which were measured.

Although it is not desirable here to give any general account of the procedure upon which these color determinations have been based, it is worth while to explain briefly the derivation of the four values ('black,' 'white,' 'color' and 'R:G') which are included in the tables. It is necessary first to state that the portion of the pelage under consideration<sup>4</sup> is examined through three color screens successively, in comparison with a 'standard' block of white magnesium carbonate. The reading for the skin, in each case, is expressed as a percentage of the value for the standard. The difference between the highest reading (here that for red) and 100 per cent is regarded as being the percentage of 'black.' The lowest reading (that for the blue-violet) is regarded

<sup>4</sup> Equal areas, similarly situated, are used in all cases.

as expressing likewise the percentage of 'white,' while the difference between the lowest and the highest readings is regarded as representing the proportion of 'free' color, i.e., of the light remaining after deducting the white. The 'R:G ratio' is the ratio between the values for red and green, after deducting from each the value for white. In a general way this ratio depends upon the spectral position of the 'free' color, though needless to say the wave length of the latter cannot be derived in any direct way from these readings. The color in all cases lies somewhere between red and yellow and is much more constant in quality than the great differences in the appearance of the skins would lead one to suppose.

It has been the object, in preparing these various series of skins, to include only those which were in the final or 'adult' phase of the pelage. For the wild stocks this was accomplished by keeping animals, believed to be fully adult when trapped, for several additional months in captivity before killing. In the case of the first two hybrid series, killing was postponed until an age of between six and one-half and seven months. Some preliminary observations led me to suppose that animals at that age could be depended upon to be in fully mature pelage. This was later found to be unwarranted. Some of the skins in both of the first two hybrid series (particularly in the Eureka-Victorville one) were found to be in the transition from the post-juvenile to the adult condition. The results from these are therefore to be accepted with some reservation.

In justification of including these not quite mature skins, it may be said, 1) that the racial differences, as well as the more pronounced individual variations of a genetic nature, commonly far outweigh the difference between the post-juvenile and mature pelages of the same animal. 2) Both hybrid generations, in each of these crosses, were killed at the same age, so that the degree of immaturity is probably closely alike in the two cases. 3) The parent-offspring correlations ( $F_1 - F_2$ ) for these color characters, are even greater in the case of the Carlotta-Calistoga cross than in the Carlotta-Victorville one, although in the latter lot there is no question as to their maturity. This is sufficient evi-

dence that the proportion of 'genetic' variability in the total variability has not been seriously diminished in the former series. As regards the Eureka-Victorville cross, on the other hand, both the parent-offspring and fraternal correlations for the coat-color characters are very low, and this may well be due to a large proportion of non-genetic variability due to incomplete molting.

The Carlotta-Victorville animals, of both hybrid generations, were reared to a more advanced age before killing, so that we are not here concerned with differences due to immaturity of pelage. Likewise, in the case of these animals, the possibility of seasonal variation in pelage was eliminated by killing all of the animals at the same time of the year (late March and early April). This, however, resulted in the animals differing rather widely in age. Those of the  $F_1$  generation varied from rather less than nine months to more than ten months old. In the  $F_2$  generation, the 'earlier broods' ranged from fifteen to nineteen months in age, while the 'later broods' ranged from twelve to nearly fourteen months.

Owing to the difference, just referred to, between two sections of the  $F_2$  material in this cross, it has been regarded as advisable to deal with these two sections separately in our statistical treatment. This is the more necessary, owing to the difference in the conditions to which these two lots of mice were subjected. The 'earlier' broods were reared, like all the rest of our stock, in a special building ('murarium'), unheated, and freely ventilated. The 'later' broods were reared in the basement of the library building of the Scripps Institution, where they were kept at a somewhat higher, as well as a much more constant temperature. As a possible consequence of this treatment,<sup>46</sup> the 'later' broods consisted, on the average, of slightly larger animals, having all of the linear measurements somewhat greater than those of the 'earlier' ones, and having an appreciably lower variability. This last fact makes it seem probable that the variability of all these hybrids (of both generations) is due, in some measure, to external conditions.

<sup>46</sup> The age of the parents may, however, have played a part in these results.

No detailed discussion seems worth while of the differences in the mean values of the various characters in the two hybrid generations of all the crosses here considered. As regards the linear measurements, we have other evidence that they are affected by metabolic differences among the individual organisms, as well as by racial differences of a genetic sort. In some instances, they are known to be affected by differences in the atmospheric temperature.<sup>47</sup> In the present tables, the  $F_1$  and  $F_2$  means are seen to differ at times rather widely from one another, in cases where no satisfactory explanation can be offered (e.g., tail length in table 3, and ear length in all three of the tables). Since those characters which are subjected to linear measurement (including tail stripe) are all ones which are modified (diminished) by the conditions of life in captivity, it is of interest for present purposes, to ascertain whether there is more of this modification to be detected in one hybrid generation than another.

Accordingly, I have compared the two hybrid generations of each cross with respect to the mean values of the following characters: tail per cent, foot (male), foot (female), femur, skull and tail stripe. The differences ( $F_2 - F_1$ ) have been computed for each. Without discussing in detail the various characters, it may be said that of the thirty-six differences here represented (the two  $F_2$  lots being treated separately) twenty-nine are negative and seven positive. In other words, in twenty-nine of these cases, the mean value for the  $F_2$  animals is smaller.

It might accordingly be contended that the  $F_2$  animals were less normal (more modified) than the  $F_1$ , and that this fact is accountable for the prevailing increase of variability in the second generation of hybrids. That such an inference cannot fairly be drawn seems plain from the following facts:

1. The increase of variability in the  $F_2$  generation does not relate preponderantly to those characters which show this reduction of size. An association table (not here reproduced) which I have constructed to test this point reveals an almost complete lack of association between the sign of the difference between the means and that of the difference between the standard deviations.

<sup>47</sup> E.g., in my own experiments with white mice.

2. In respect to half of the characters mentioned above, the mean  $F_1$  value exceeded the mean of the values for the two parent races. In certain cases, indeed, it exceeded both of the parental

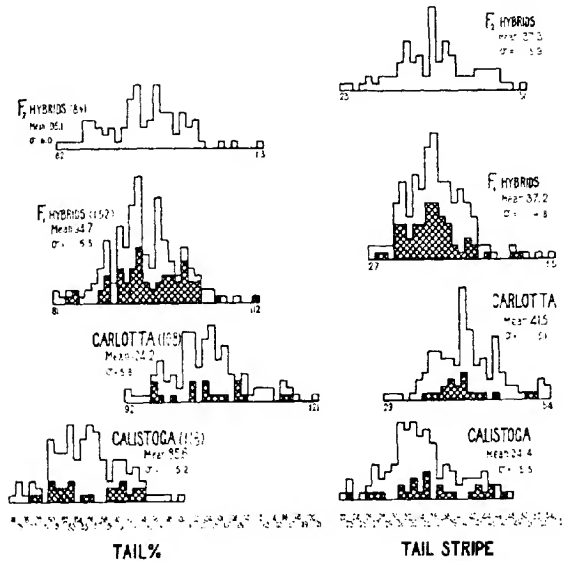


Fig. 1. Showing the range of variability in the Carlotta and Calistoga races and in  $F_1$  and  $F_2$  hybrids. (Modified, from Sumner, Jour. Exp. Zool., '20.) The left-hand series of histograms is based upon relative tail length; the right-hand ones upon width of tail stripe. Cross-hatched areas in the pure races represent parents of  $F_1$  broods. Such areas in the  $F_1$  represent parents of  $F_2$  animals, together with the sibs of such parents. Figures in parenthesis represent the number of individuals comprised in the series in question.

values. It is quite possible that this is a manifestation of heterosis or 'hybrid vigor.'<sup>48</sup> Thus the reduction in the relative size of a part when we pass from the  $F_1$  to the  $F_2$  generation does not in all cases represent a decrease in relation to the mid-

<sup>48</sup> The extreme size and weight of some of the  $F_1$  mice lends support to this interpretation. My results, in this regard, are quite similar to those of Castle (Carnegie Inst. Publ. No. 320, 1922).

parental value. The mean  $F_2$  figures for foot length (both male and female) remain higher than the parental means for this character, while the figures for ear and skull are almost identical with the mid-parental ones.

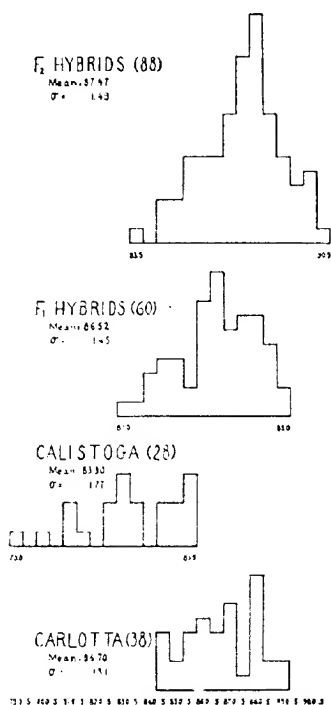


Fig. 2 Histograms based upon the values for 'black' in the pelage of the Carlotta-Calistoga series.

3. I have compared mean values and standard deviations for several of the 'pure' races, in the wild and in the first and second cage-bred generations. The important point derived from this comparison is the fact that, in respect to all three of the characters chosen (tail per cent, foot length, and tail stripe) the variability was actually less in the second than in the first cage-born generation, although the mean value of the measurements was smaller

in two cases out of three. So far as may be judged from these figures, therefore (and they are based upon 237 second-generation mice), there is no evidence that variability increases in successive generations of captive mice.

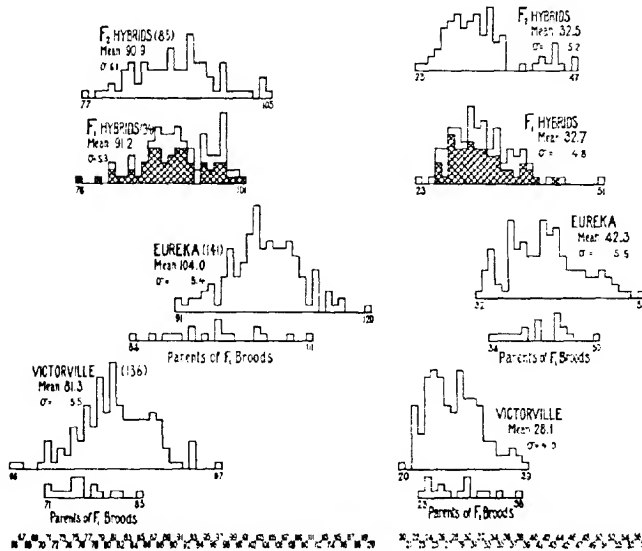


Fig. 3. Histograms for relative tail length (left) and width of tail stripe (right) in the Eureka-Victorville series. (Modified from Sumner, Jour. Exp. Zool., '20.) The cross-hatched areas in the  $F_2$  generation represent parents of  $F_2$  broods, together with the sibs of such parents. The actual 'pure' race parents of the  $F_1$  hybrids were in this case cage-bred animals. They are indicated below the larger histograms for the pure races, which represent more accurately the character of these races.

The upshot of the present discussion seems to be that the  $F_2$  hybrids dealt with in the present experiments are more variable than the  $F_1$ , for the reason that they are  $F_2$  hybrids and not because of extraneous circumstances.

It is worth mentioning, in respect to the color characters, that in two of the crosses out of three the value for black in the  $F_1$  generation was greater than the mid-parental value (the values

for white showing a reverse relation) while in two cases out of three the  $F_2$  value was greater than the  $F_1$  (figs. 2, 4, 6). For this reason, in the Carlotta-Calistoga cross, of both generations, and the Carlotta-Victorville cross, of the  $F_1$  generation, the number of individuals falling within the range of the darker sub-

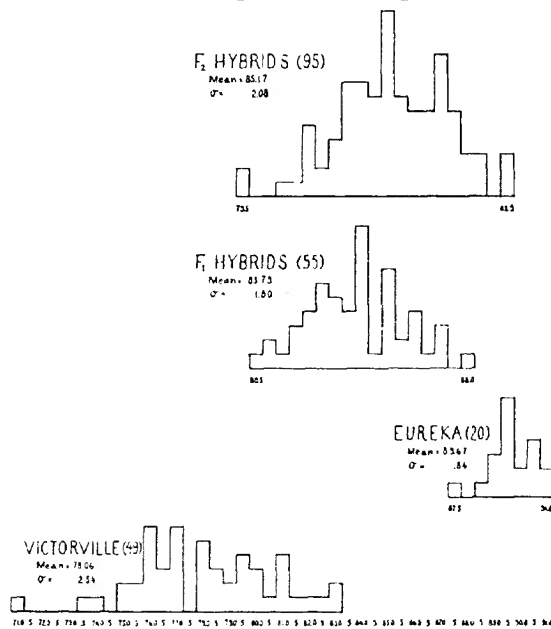


Fig. 4 Based upon percentages of 'black' in the Eureka-Victorville series

species greatly preponderates over the number resembling the paler subspecies. It is likewise to be noted (fig. 6) that the 'later'  $F_2$  broods of the Carlotta-Victorville cross are darker than the 'earlier' ones. Since these last two series differ in respect to age, it seems possible that age may be responsible for some of these differences. I have encountered other evidence that older mice tend to be slightly paler than younger ones, even when the latter are in the 'adult' pelage phase.

Some explanation is demanded of the table (table 4) comprising the parent-offspring and fraternal correlations. In computing the former series of coefficients, the parental deviation, in each case, was repeated as many times as there were offspring belonging to each parent, the total number of offspring being the 'n' of the

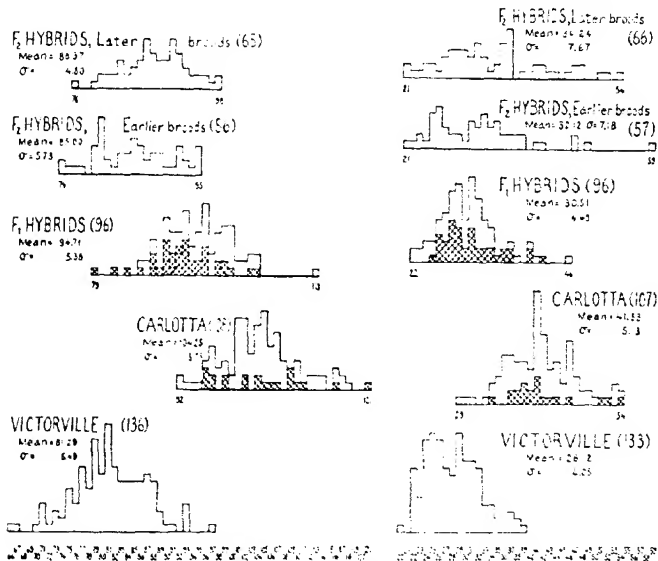


Fig. 5 Histograms for relative tail length (left) and width of tail stripe (right) in the Carlotta-Victorville series. For explanation see figure 1. No cross-hatched areas are included in the Victorville polygons, since the actual parents belonged to a limited series not here comprised. It is important to state that a few of the most extreme values for the tail stripe are perhaps not comparable with the others (see text).

formula. In computing the probable errors for these coefficients, on the other hand, a lower value of 'n' was employed. This was the arithmetic mean between the number of offspring and the number of parents. These last two numbers, connected by hyphen, are the ones which appear in the columns headed 'numbers' in the upper half of the table. It will be seen that the numbers upon which the coefficients for the color characters are

based are in some cases considerably smaller than the others (for reasons see p. 280).

In the case of the Carlotta-Calistoga and the Carlotta-Victorville crosses, there are given both the correlations between the parent races and the  $F_1$  hybrids and the correlations

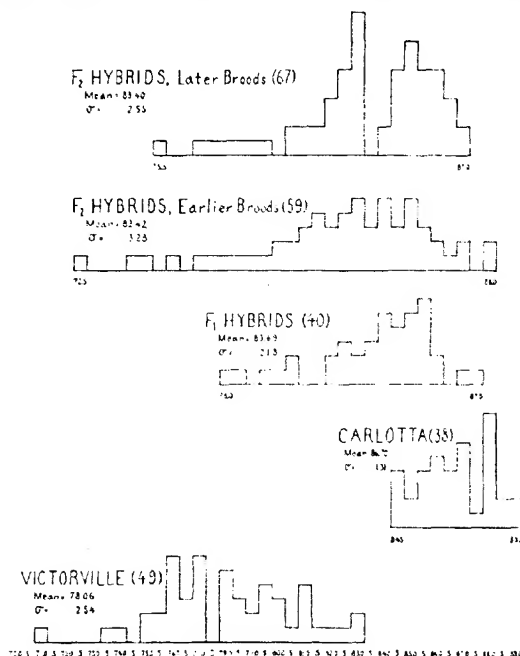


Fig. 6 Based upon percentages of 'black' in the Carlotta-Victorville series

between the latter and the  $F_2$  hybrids. In the case of the Eureka-Victorville cross, the former set of correlations are lacking, owing to the fact that the animals of the P generation were in this case not measured. They were all cage-bred animals.

In computing the parent- $F_1$  correlations, the two races have been dealt with separately, though the sexes have been thrown together. This last procedure is permissible, in as much as we are

TABLE 4  
*Parent-offspring and fraternal correlations*

	TAIL (PERCENT)	TAIL STRIPS	FOOT PROMINENCE	SNM	BLACK	WHITE	COLOR	MEANS	R:O	NUM- BERS
Parent-offspring										
Carlotta-P...	+0.04±0.08	+0.12±0.07	+0.14±0.07	118.20	+0.20±0.11	+0.37±0.10	+0.08±0.12	-0.197	-0.04±0.12	54-11
Carlotta-P...	+0.47±0.06	+0.31±0.07	+0.21±0.07	110.26	+0.24±0.11	-0.06±0.12	+0.33±0.11	+0.06±0.11	+0.06±0.11	52-10
P <sub>1</sub> -P <sub>2</sub> (fathers)	+0.25±0.09	+0.35±0.09	+0.31±0.09	84.14	+0.42±0.08	+0.10±0.09	+0.41±0.08	-0.332	+0.16±0.09	86-14
P <sub>1</sub> -P <sub>2</sub> (mothers)	+0.41±0.08	+0.25±0.09	+0.21±0.09	80.21	+0.44±0.07	+0.13±0.09	+0.49±0.07		-0.08±0.09	88-24
P <sub>1</sub> -P <sub>2</sub> (fathers)	+0.42±0.08	+0.32±0.09	+0.43±0.08	86.13	-0.02±0.10	+0.20±0.09	-0.10±0.09	+0.103	-0.01±0.10	82-14
P <sub>1</sub> -P <sub>2</sub> (mothers)	+0.50±0.07	+0.09±0.10	+0.43±0.07	90.27	+0.13±0.09	+0.13±0.09	+0.24±0.08		+0.18±0.08	90-27
Carlotta-F <sub>1</sub>	+0.16±0.09	+0.11±0.09	+0.18±0.09	95.23	-0.11±0.11	-0.07±0.13	-0.28±0.13	+0.154	+0.18±0.13	38-6
Victorville-F <sub>1</sub>	+0.65±0.09	+0.51±0.07	+0.52±0.08	97.21	+0.40±0.11	+0.34±0.13	+0.47±0.11		-0.25±0.13	31-10
P <sub>1</sub> -P <sub>2</sub> (fathers)	+0.22±0.08	+0.34±0.08	+0.47±0.06	121.11	+0.03±0.08	+0.38±0.07	-0.03±0.08		+0.14±0.08	118-10
P <sub>1</sub> -P <sub>2</sub> (mothers)	+0.37±0.08	+0.21±0.08	+0.33±0.08	104.17	+0.15±0.07	+0.22±0.08	+0.46±0.07	+0.217	+0.18±0.08	110-18
Means (weighted)	+0.284	+0.258	+0.298		+0.231	+0.200	+0.220	+0.217	+0.099	
Fraternal										
Carlotta-Carlotta, F <sub>1</sub>	+0.55±0.04	+0.51±0.04	+0.36±0.05	118	+0.51±0.05	+0.51±0.08	+0.49±0.07	+0.437	-0.40±0.09	60
Carlotta-Carlotta, F <sub>2</sub>	+0.39±0.06	+0.37±0.06	+0.43±0.06	85	+0.40±0.06	+0.37±0.06	+0.38±0.06	+0.383	+0.08±0.07	85
Carlotta-Victorville, F <sub>1</sub>	+0.42±0.06	+0.32±0.06	+0.54±0.05	100	+0.11±0.09	+0.33±0.08	+0.17±0.09	+0.212	-0.06±0.09	54
Carlotta-Victorville, F <sub>2</sub>	+0.78±0.03	+0.55±0.07	+0.43±0.06	90	+0.02±0.07	+0.09±0.07	+0.51±0.07	-0.095	+0.17±0.07	80
Carlotta-Victorville, F <sub>3</sub>	+0.31±0.06	+0.48±0.05	+0.52±0.05	90	+0.02±0.07	+0.37±0.09	+0.52±0.08	-0.303	-0.17±0.11	38
Carlotta-Victorville, F <sub>4</sub>	+0.57±0.04	+0.41±0.06	+0.53±0.04	119	+0.52±0.05	+0.58±0.04	+0.47±0.05	+0.523	-0.10±0.06	119
Means (weighted)	+0.514	+0.351	+0.406		+0.357	+0.351	+0.351	+0.359	-0.011	

dealing with characters which show no sexual differences. For the  $F_1$ - $F_2$  correlations, on the other hand, the sexes have been dealt with separately.

The figures in the column headed 'means' are the weighted means of the three color characters ('black,' 'white,' and 'color'), and for the two sets of figures (e.g., Carlotta- $F_1$  and Calistoga- $F_1$ ) representing each type of correlation.

In the computation of the fraternal correlations, the deviation for each member of a brood was paired off with the deviation

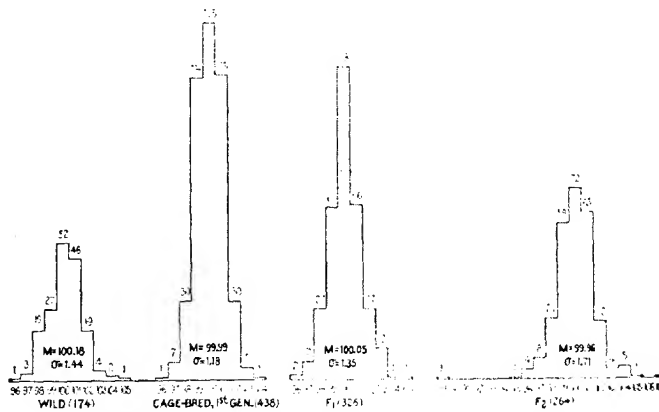


Fig. 7 Based upon dextro-sinistral ratios for the two halves of the lower jaw, in wild mice of several 'pure' races, cage bred animals of the first generation (likewise of 'pure' race), and  $F_1$  and  $F_2$  hybrids (three series combined). The abscissas represent the ratios, the ordinates standing for the frequencies of each. (Sumner and Huestis, Genetics, '21 )

of the mean value of its sibs. The 'n' employed, both for the correlation coefficients and the probable errors, was the total number of animals comprised in the generation dealt with. This method of procedure has some statistical disadvantages, but it seemed the best one to employ in the present circumstances. The figures in the columns headed 'numbers' represent the total number of individuals of each generation upon which the coefficients in question are based.

## EXPERIMENTS ON THE REVERSAL OF THE SPINAL CORD IN AMBLYSTOMA EMBRYOS AT THE LEVEL OF THE ANTERIOR LIMB<sup>1</sup>

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TWENTY-FIVE FIGURES

### INTRODUCTION

From the results of previous experiments involving the transplantation of the anterior limb and the corresponding level of the spinal cord in *Amblystoma* embryos, a number of considerations were directed towards an analysis of certain morphogenetic and functional readjustments of the central and peripheral nervous system in response to the altered conditions.

One of the outstanding results of the limb experiments was the demonstration of a remarkable propensity on the part of the normal brachial nerves to supply the limb when the latter was transplanted short distances (from one to three segments) caudal or rostral to its normal site. This tendency for the displaced limb to receive the normal nerve supply was not interpreted as a definite specific relation between these nerves and their normal terminal musculature, for it was found that limbs, which were reimplanted at greater distances than above mentioned, received their nerve supply from plexuses formed by nerves which normally supply the abdominal musculature.

It became perfectly clear in these experiments that the extent to which a transplanted limb could function depended primarily upon the amplitude of its nervous connection with the normal brachial reflex mechanism existing within the central nervous

<sup>1</sup> The experiments were carried out at the Anatomical Laboratory of the Yale School of Medicine in the spring of 1920.

system. Limbs receiving their nerve supply from spinal segments lying beyond the limits of the normal brachial level, were found incapable of perfect coördinate movements, even though in the possession of complete skeletal and muscular differentiation and an adequate intrinsic supply of nerves.

In the light of the apparent 'preference' on the part of the brachial nerves for their normal end organ (limb) when the latter occupied a heterotopic position, it became of interest to determine if possible whether or not in normal development there is any evidence of an intimate developmental relationship between a given nerve and the particular muscles which it supplies, or whether the developmental reactions on the part of the brachial nerves towards their displaced end-organ are of more general nature, i.e., referable only to the shoulder and limb as developmental systems.

Previous experiments (Braus, '05; Harrison, '07, and Detwiler, '20 b) have shown that, regardless of the segmental nerve contribution, the intrinsic nerve distribution within a transplanted appendage is exactly the same as in the normal. That this is subsidiary to the mode of segregation of the structures within the differentiating appendage, was pointed out by Harrison (*op. cit.*). From this we see that, whereas the topographical relations of the differentiating structures within the end-organ undoubtedly play an important rôle in determining the final path of the nerve fiber, such mechanical influences do not reveal the mechanism whereby the initial connection between the nerve fiber and its particular end-structure is established. The plausibility of the concept that peripheral selectivity may be determined by an electro-chemical mechanism such as offered by Kappers ('17) in explanation of the dynamic polarization of the neurone and selectivity within the central nervous system has been suggested previously (Detwiler, '23).

By reversing the limb level of the spinal cord, the normal relationship between the outgrowing nerves and elements of the muscle blastema is entirely upset and a condition is presented whereby the forces which underlie normal connections in this region can be studied further.

Under normal conditions of development the muscles connecting the shoulder girdle with the limb and also the limb muscles receive their total nerve supply from a plexus derived from the third, fourth and fifth spinal nerves ('20 b, fig. 2). The third nerve, although contributing a few fibers to the extensor nerves of the limb, supplies the major portion of its motor fibers to the shoulder muscles (*op. cit.*, p. 128). The fourth nerve also contributes some fibers to the shoulder muscles, but the greater portion of this nerve enters the limb whereas the fifth nerve, which is considerably smaller than the third or fourth, appears to contribute an approximately equal supply to both shoulder and limb. The source of the nerves to the various muscles connecting the limb with the shoulder is given in table 2.

From the results of previous spinal cord transplantation experiments considerable evidence has been produced to show that the extent of motor cellular differentiation within a given region of the spinal cord does not depend upon the functional demands of the terminal musculature, but that it is more directly influenced by stimuli coming from descending longitudinal pathways with which functional connection is established. In these experiments it was found that the motor elements in segments of the spinal cord located caudal to the limb level did not undergo any hyperplastic development when subjected to the added functional stress of a terminally increased musculature (limb and shoulder muscles). However, when these same segments were substituted for the anterior limb level of the cord, they underwent hyperplasia approximately equal in extent to the increased development which normally characterizes the brachial enlargement. From these results, it became apparent that the typical increased proliferation of the primary brachial motor neurones is not primarily due to the demands of the limb musculature, but rather as the result of the increased stimulation emanating from the longitudinal conduction pathways which normally terminate at this level of the cord and are concerned in the coördinate control of the limb musculature. This interpretation was further supported by the results of limb extirpation experiments in which it was shown that the excision of the limb rudiment had no measurable effect upon

the early extent of differentiation of the primary brachial motor neurones, as was evidenced by comparing the weight, size, and number of nerve cells in the two halves of the spinal cord at the limb level (Detwiler, '23, figs. 7, 8, and 11; also tables 2, 4, and 5).

Under normal conditions of development, the brachial portion of the cord (third, fourth and fifth spinal segments) exhibits a gradual decrease in size, and number of cells as we pass from its rostral to its caudal limit. In all of the previous experiments, viz., those in which the limbs were excised as well as those in which a more caudal unit of cord was substituted for the normal limb level; the size, volume, and cellular relations throughout this level remained constant (the rostral portions undergoing a greater degree of differentiation than the caudal), and it became of further interest to determine whether or not this same relation would persist if the limb level of the cord were reversed. The maintenance of normal developmental relations under these conditions would thus serve as another corroborative point of evidence favoring the view that the extent of differentiation of the primary brachial motor neurones within a given segment is determined primarily by the number of longitudinal fibers normally terminating at that level.

#### EXPERIMENTAL

Embryos in two stages of development were employed in the experiments, viz.; those with completely closed neural folds (stage 21 to 23), and those with a prominent tail-bud (stage 29 to 30). Transverse incisions were made across the spinal cord at the levels corresponding to the notches between the second and third, and the fifth and sixth somites. Two paramedial longitudinal incisions were then made between the spinal cord and the somites, care being taken to include as little of the somites as possible. The sectioned unit of cord was then carefully freed from the underlying notocord, which was left undisturbed, and reversed end for end in the same animal. Following the operation, the embryos were kept at 15°C. for several days, after which they were allowed to develop at room temperature.

The effects of the reversal upon the general development of the embryo, the developing swimming reactions, and the functional behavior of the limbs are summarized in table 1. Although the number of positive experiments is small, they are sufficient to indicate that a greater percentage of normal results were obtained when the younger embryos were used for the operation. This was also found to be true in the experiments, recently reported, in which the anterior limb level of the cord was excised and replaced by a more caudal unit of cord.

TABLE 1

*Showing the effects of reversing the anterior limb level of the spinal cord (third, fourth, and fifth spinal segments)*

SERIES	STAGE OF DEVELOPMENT WHEN OPERATION WAS PERFORMED	NUMBER OF OPERATIONS		FOUR- TILE EX- PERI- MENTS		NOR- MAL CASES		ABNORMAL CASES						INITIAL TOTAL REACTIONS		LIMB REACTIONS				
								Kyphosis		Lordosis		Scoliosis		Perfect	Im- perfect	Normal	Ab- normal			
		Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent			
		Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	
A	21-23	75	15	20	10	66	1	7	1	7	3	20	10	67	5	33	15	100	0	0
B	29-30	50	4 <sup>1</sup>	8	1	25	3	75						0	0	4	100			

<sup>1</sup> All four cases died prior to period when limb reactions begin.

Wieman ('22), who used corresponding ages in his neural tube transplantation experiments, claims that lordosis is apt to be more marked when the earlier stages are employed. He also suggests that kyphosis is more prevalent in those cases in which the notocord was left intact and that in those cases where it was transplanted with the neural tube, kyphosis was slight or even absent. In my own spinal cord experiments (both transplantation and reversal), kyphosis was found to increase with the age of the embryo at the time of the operation ('23, table 1; and table 1 of this paper). It thus seems doubtful from my own observations that this abnormal posture, which many embryos assume in later development can be referable to the notocord. It would seem that subsequent abnormal postures of the embryos should more likely follow in those cases in which the notocord

had been sectioned rather than in those in which it has been undisturbed. Even in the limb transplantation experiments, in which no damage has been done either to the neural tube or the notocord, a certain percentage of the larvae develop kyphosis.

Wieman (*op. cit.*) thinks that lordosis which he found to be more prevalent when using young stages, results from contraction accompanying closure of the wound. In my own experiments, though only a very few individuals developed this condition, it did not appear until seven to eight days after the operation whereas the initial reactions began approximately four days after this period. In all of the cases the wound was well healed over and the animal had assumed an absolutely normal posture for several days prior to the onset of the defect. The fact that both kyphosis and lordosis have developed in embryos used exclusively for limb transplantation work, suggests that the causative factors underlying these curvatures are not as yet clear.

Twenty per cent of the cases (three in fifteen) with reversed spinal cords developed scoliosis (table 1). In two cases, the lateral abnormal curvature did not appear until twenty days after the operation and was preceded by kyphosis. These abnormal individuals were not fixed until approximately fifty days after the operation, and only one has been studied in section (case ReSe 11). Although the sections do not reveal the cause of the curvature, they do show that the spinal cord and the nerve roots are normally developed and that there is perfect continuity of the notocord and vertebral column. These later, however, are markedly deflected from the median longitudinal axis. From the medulla caudal to the level of the sixth segmental nerves, this curvature is towards the right (fig. 13). From this region caudal to almost the posterior limb level, the deflection is towards the left (fig. 14). It was at first thought that the lateral curvature might result from not reimplanting the reversed piece in absolute alignment with the intact portions of the cord, yet this is undoubtedly not the case, for in Wieman's experiments (*op. cit.*) the sectioned piece of cord was reimplanted perpendicular to the original axis, yet apparently scoliosis did not result as a distinct abnormality in his experiments. The scoliotic condition which

resulted from these experiments may be the result of injury inflicted upon the sclerotome by the operation though in the experiments in which the limb level was excised and replaced by a strange unit of cord, the embryos were subjected to the same type of injury and yet no cases of scoliosis resulted.

Since it was desired to keep the operated larvae for a considerable time in order to study the reactions of the limbs, no sections were made of embryos during the period of wound healing and the early reaction stage. The steps involved in healing and the method of reunion of a sectioned unit of cord with the intact portions under the conditions of complete end to end reversal and 90° rotation on the longitudinal axis, have been described and discussed by Hooker ('15, '17) and by Wieman ('22). One of the outstanding observations in Hooker's reversal experiments was the marked tendency for the developing nerve fibers (both sensory and motor) to avoid entering the wound surfaces opposite them. According to him, it was this avoidance of the opposite wound surfaces chiefly on the part of the sensory fibers which caused the failure of so many of the embryos with 'primarily' unfused wounds to reestablish continuity of the cord, and indeed to live.

From his experiments in which a unit of cord was rotated through 90° on its longitudinal axis, Wieman (op. cit.) found that union between the caudal end of the cephalic stump and the original cephalic end of the rotated portion readily takes place by motor fibers growing caudally into the rotated piece. The cephalic end of the caudal stump, however, makes no attempt to unite with the caudal end of the rotated piece until motor connections cephalo-caudad through the rotated piece with the caudal stump is established. Once the stumps have been bridged by descending fibers, all semblance of 'repulsion' disappears, and the way is prepared for ascending sensory tracts to develop. Wieman (op. cit., p. 182) is of the opinion that complete fusion of the grafts with the intact stumps does not occur until nerve fibers develop, as evidenced by the fact that in his 'G' series, which were operated upon just before the onset of reactions, the connections between the transplant and the intact stumps de-

veloped in a much shorter time than in those which had been operated upon in earlier stages. He also claims that, when the rotated unit of cord was taken from a more caudal level (region of the fifth and sixth somites), the restoration of nervous continuity through the sectioned piece could not be demonstrated during the first thirty days, and he concludes that the nearer the brain the transplantation is made, the better are the chances for connection to be established.

In my own transplantation experiments (both those in which the anterior limb level was reversed, and those in which this same level was excised and replaced by a more caudal unit of cord), the greater percentage of cases which exhibited perfect initial responses to tactile stimulation were those which had been operated upon when in the earlier stages. In the larvae with composite spinal cords, 53 per cent of those which were operated upon in the tail-bud stages exhibited imperfect reactions, whereas all surviving cases which were operated upon just after the closure of the neural folds, passed through the developing reaction stage without showing the least signs of any defective reactions resulting from incomplete union. In the spinal cord reversal experiments only 33 per cent of those operated upon in the earlier stages showed imperfect reactions to stimulation, whereas all those operated upon in the tail-bud stage gave defective reactions. These results indicate that perfect anatomical and functional union take place much more readily when the younger stages are employed in the operation. In all these experiments the involved level of the cord included the third, fourth and fifth segments— a level only slightly more rostral than that used in Wieman's so-called 'F' series in which he found that continuity through the sectioned piece did not take place within the first thirty days, yet in my own experiments initial reactions began approximately six days after the operation. In the majority of cases these developed normally, and perfect serpentine swimming reflexes were executed long before the period when limb reactions began (from seventeen to twenty days). From this it would appear that union of the sectioned piece with the intact stumps is not necessarily dependent upon the caudal growth of fibers from the

cephalic through the sectioned piece, but that in the cases where the young embryos are employed, complete healing *per primam intentionem* ensues. Hooker ('15, '17) showed that this was the case in his experiments on the frog embryos, and that only in those cases where the wound surfaces were separated did development of the nerve tracts play an important rôle in bringing about union between the sectioned piece and the intact stumps. The development of the nerve fibers from the cephalic stump through the sectioned piece of cord may have been the method whereby union was effected in Wieman's experiments since in all of his experiments the rotated unit of cord carried with it some muscle blastema and the developing muscle thus formed a non-nervous block between the rotated unit of the cord and the intact stumps. This method of healing, as he observed, corresponds to that which Hooker (*op. cit.*) observed in his cases where the reversed piece was secondarily separated from the intact stumps and a gap was formed. However, in end to end reversal or in uniting composite cords, initial union of the sectioned piece with the intact stumps can and does take place without the intervention of developing nerve fibers provided the surfaces are closely opposed and the operation is done well in advance of the period when the primary reflex mechanism is developed.

#### REACTIONS OF EARLY EMBRYOS TO TACTILE STIMULATION

Although the developing reflexes in these experiments were not studied as carefully as in the animals with composite cords, it is seen from table I that the greater percentage of animals with perfect initial responses were those which had been operated upon when in the early stages. When the operation was performed on embryos just prior to the stage when reactions begin (stage 29 to 30), the four embryos of this series showed imperfect reactions. None of these cases showed any improvement and all died before the expiration of the tenth day. Of those which were operated upon just after complete fusion of the neural folds, five cases exhibited imperfect initial responses to tactile stimulation (table I). These improved and later developed normal serpentine swimming reflexes.

The character of the defect in those with imperfect reactions was, in the main, the same as that observed in embryos with composite cords (Detwiler, *op. cit.*). Imperfect conduction through the reversed piece, both caudo-cephalad and cephalo-caudad was evidenced, although the ascending sensory conduction on the whole showed more improvement than descending motor conduction, which, in a few cases remained perceptibly impaired up to the time of death of the embryo. In those which survived, perfect conduction in both directions was attained. The more general extensive impairment of the motor conduction over the sensory, which a few cases showed, was attributed chiefly to a more extensive injury inflicted upon the ventral portion of the cord during the operation—since the manipulation in freeing the cord from the underlying notocord (which was left intact) frequently resulted in considerable damage to the floor of the neural tube.

#### REACTIONS OF THE LIMBS

From an examination of table 1 (A), it is seen that in all of the positive cases, the limb reactions were normally executed. Even in those which developed the various abnormal postures and in which swimming reactions were later impaired by these curvatures, the limbs showed no defects in function. It should be noted in table 1 that the five cases with imperfect initial total responses do not correspond to the five cases with various spinal curvatures, for in all cases this latter defect did not appear until after the initial swimming reflexes had been developed. The method of testing the limb reflexes and the criteria employed in establishing the category 'normal function' was the same as previously used (Detwiler, '20 b, pp. 131-132).

Five of the animals including one case with marked scoliosis were sectioned and a study was made of the brachial nerves and the limb level of the cord. The sections were cut transversely at  $10\mu$  and were stained with Ehrlich's haematoxylin and erythrosin.

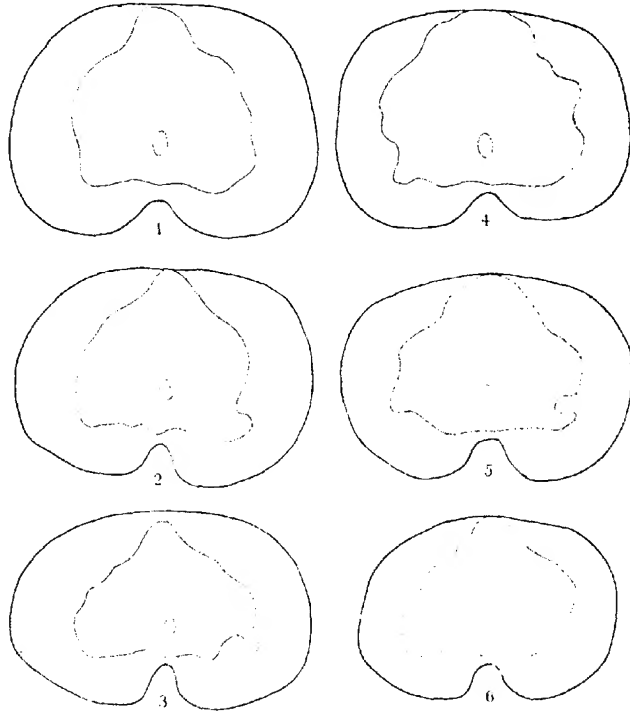
## DESCRIPTION OF CASES

*Case ReSc 1.* This case was operated upon when in stage 23. Wound healing was rapid and the developing reflexes proceeded normally. Limb movements appeared twenty-five days after the operation. This individual, both as regards external appearances and behavior, could not be distinguished from a normal animal. It was photographed fifty-three days after the operation just prior to its fixation. The photograph is seen in figure 15.

The internal findings in this case were exactly as in a normal animal both as regards the distribution and peripheral connections of the nerves as well as the degree of development of the spinal segments contributing these nerves.

As has been stated above, under normal conditions of development, the limb muscles and those connecting the shoulder girdle with the limb, are supplied by a plexus derived from the third, fourth and fifth spinal segments. Under the conditions of the experiment, the fifth spinal segment occupies the region of the original third whereas the third occupies that of the original fifth. The fourth remains constant. The source of the nerves to the various shoulder-limb muscles under normal and experimental conditions is given in table 2. From this we see that the interchanged fifth and third segments are carrying out, respectively, the functions of the normal third and fifth. Under normal conditions of development, the ventral ramus of the fifth nerve is considerably smaller than that of the third, whereas under the experimental conditions the ventral ramus of the third (reversed fifth) shows the typical increase in size over the fifth (reversed third). An idea of the relative sizes of the normal third, fourth and fifth segments of the cord can be obtained from figures 1, 2, and 3 which represent typical sections passing through these respective levels, and also in the models shown in figures 17, 18, and 19. These size relations are always constant - the third segment being larger than the fourth, and the latter in turn larger than the fifth. The size relations of these segments under the experimental conditions in this case are shown in figures 4, 5, and 6 (cf. figs. 1, 2, 3) and in figures 20, 21 and 22 (cf. figs.

17, 18, and 19). These are seen to compare favorably with the normal. The third segment (reversed fifth) is larger than the fourth, and the latter is larger than the fifth (reversed third). The extent of cellular differentiation within the cord and the spinal ganglia at these levels, under the normal and the experimental conditions, is shown in tables 3 and 4, respectively.



Figs. 1, 2, and 3 Typical transverse sections of the spinal cord at the levels of the third, fourth, and fifth nerves, respectively (first, second and third brachial nerves), in a normal *Amblystoma* larva of 68 days (AS4).  $\times 100$ .

Figs. 4, 5, and 6 Typical transverse sections of the spinal cord at the levels of the third, fourth, and fifth nerves, respectively (first, second and third brachial nerves), in operative case ReSe 1. Limb level of the spinal cord (third, fourth and fifth segments) reversed. Animal preserved 53 days after operation.  $\times 100$ . (Cf. figs. 1, 2, and 3.)

TABLE 2  
Showing the muscles connecting the shoulder girdle with the anterior limb and their respective nerve supply.

CONDITIONS	CASA	MUSCLES					
		M. pector. gros. lateralis	M. subcoraco- scapularis	M. supra- coracoclavicularis	M. coracohumeralis supraclavicularis	M. latissimus dorsi	M. pectorales
Normal.....	ASL 1	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)
Anterior limb held of spinal cord 7, 4 and 5.	These 6	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)
segmental retracted.	These 10	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)
	These 11	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)
	These 13	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)	n. pector. 3) n. subscap. 3)

n. pector. n. pectoralis  
n. subscap. n. subscapularis  
n. supraclavicularis  
n. subcoraco- n. subcoracoclavicularis  
n. supraclavicularis  
n. lat. dorsi, n. latissimus dorsi  
n. lat. dorsi, n. latissimus dorsi  
n. cor. br., n. coracohumeralis  
n. pectoralis  
n. pectoralis  
(3), from spinal nerve III  
(4), from spinal nerve IV  
(5), from spinal nerve V

1 For normal plexus and brachial plexus, v. Dawbarn, '201, figure 2, p. 128.

TABLE 3  
*Showing number of cells counted in twenty consecutive transverse sections of the right half of the spinal cord at the levels of the third, fourth, and fifth segmental nerves respectively (anterior limb level)*

CONDITIONS	CASES	III NERVE LEVEL	IV NERVE LEVEL	V NERVE LEVEL	CELLULAR RATIO BETWEEN III AND V NERVE LEVELS
Normal	AS4	3461	2796	2346	111:V 1.48:1.00
Limb excised, spinal cord intact	AS4 <sub>16</sub>	3904	2803	Connected with limb 2352	1.66:1.00
7th, 8th, and 9th spinal segments substituted for the 3rd, 4th, and 5th, respectively	TrSC <sub>127</sub> <sup>1</sup> TrSC <sub>124</sub>	3130 3043	2600 2073	2500 2728	1.25:1.00 1.11:1.00
Limb level of spinal cord (3rd, 4th, and 5th segments) reserved	ReSC <sub>1</sub> <sup>2</sup> ReSC <sub>10</sub> <sup>2</sup>	3500 3265	2503 2809	2215 2326	1.62:1.00 1.40:1.00

<sup>1</sup> In cases TrSC<sub>127</sub> and TrSC<sub>124</sub>, the 3rd, 4th, and 5th nerve levels represent, respectively, the transplanted 7th, 8th, and 9th.

<sup>2</sup> In cases ReSC<sub>1</sub> and ReSC<sub>10</sub>, the 3rd nerve level represents the reversed 5th, whereas the 5th nerve level represents the reversed 3rd.

TABLE 4  
Showing number of cells counted in the third, fourth, and fifth right spinal ganglia

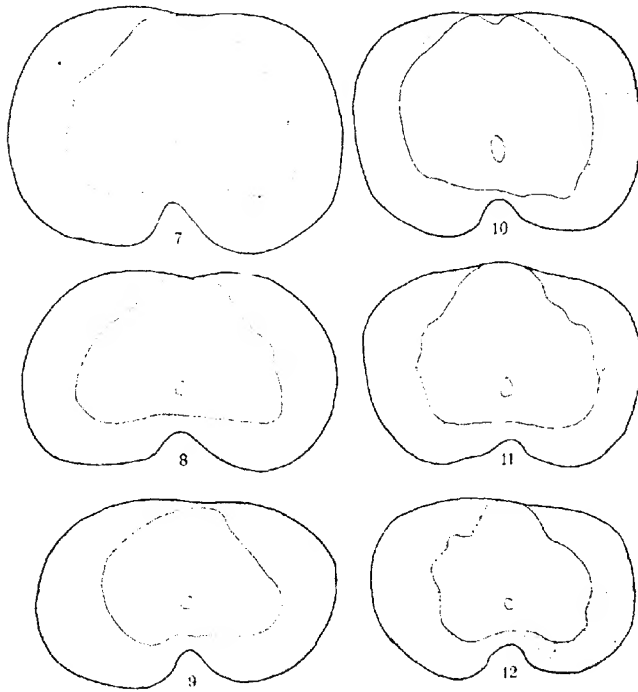
CONDITION	CASE	III GANGLION	IV GANGLION	V GANGLION	CELLULAR RATIOS III BEFORE AND V GANGLIA
Normal	AS4	1725	1430	1195	111:V 1.44:1.00
Limb excised, spinal cord intact	AS14	850	625	Connected with limb 965	0.88:1.00
7th, 8th and 9th spinal segments substituted for 3rd, 4th, and 5th, respectively	TSC17 TSC19	1186 2000	1633 1838	1270 1701	1.17:1.00 1.17:1.00
Limb level of spinal cord (3rd, 4th, and 5th seg- ments) reversed	ReSC1 ReSC10	2008 1675	2152 1451	1451 1150	1.38:1.00 1.45:1.00

It is seen from these tables that the degree of development in these reversed interchanged segments closely approximates the normal conditions both as regards cellular differentiation and size. That the motor cellular differentiation within these segments of the cord is not markedly affected by the presence or absence of the appendage is seen in table 3. With this evidence known, it is seen that the increased development within the fifth segment when substituted for the third, and the decreased development of the third when substituted for the fifth, is not a factor of their respective muscular demands. The similarity in the gradient of differentiation within the limb level of the cord under the normal and the three different experimental conditions (table 3) is significant of the existence of a stimulus other than peripheral functional demands which is primary in bringing about cellular proliferation.

The extent of sensory development, however, which has previously been shown to be secondary to the peripheral functional demands (Detwiler, '20 a, '23) does not follow as consistently the same gradient of differentiation within this level as is manifested by differentiation within the cord. Although normally there are always more cells in the third than in the fourth spinal ganglion, yet in table 4 we note that two cases under different experimental conditions show a greater differentiation in the fourth than in the third ganglion (cases TrSc 137 and ReSc 11). A corresponding cellular ratio within the cord in these cases was not found.

*Case ReSc 10.* The operation was performed upon the embryo in stage 23. Initial reactions to tactile stimulation began after five days. These were normal in character. One day later, when the embryo was in the 'S' reaction stage, slight kyphosis was noted. This increased somewhat for several days and the animal remained kyphotic up until the period when the limbs began to function (twenty days after the operation). The animal then gradually assumed normal posture. Throughout this latter period the swimming and limb reactions were normal. The animal was preserved fifty-three days after the operation.

The microscopic findings in this case were in all essentials, the same as in case ReSe 1 described above. The brachial plexus was normal as was the intrinsic distribution of its branches, the muscular connections of which are shown in table 2. In figures



Figs. 7, 8, and 9 Typical transverse sections of the spinal cord at the levels of the third, fourth, and fifth nerves, respectively (first, second and third brachial nerves) in operative case ReSe 6. Limb level of the spinal cord (third, fourth, and fifth segments) reversed. Animal preserved 58 days after the operation.  $\times 100$ . (Cf. figs. 1, 2, and 3.)

Figs. 10, 11, and 12 Typical transverse sections of the spinal cord at the levels of the third, fourth, and fifth nerves, respectively (first, second, and third brachial nerves), in operative case ReSe 10. Limb level of the spinal cord (third, fourth, and fifth segments) reversed. Animal preserved 53 days after operation.  $\times 100$ . (Cf. figs. 1, 2, and 3.)

10, 11, and 12 are shown the comparative sizes of typical sections taken respectively from the third, fourth and fifth nerve levels of the cord (cf. figs. 1, 2, and 3). The models of segments are shown in figs. 23, 24, and 25 (cf. figs. 17, 18, and 19). In tables 3 and

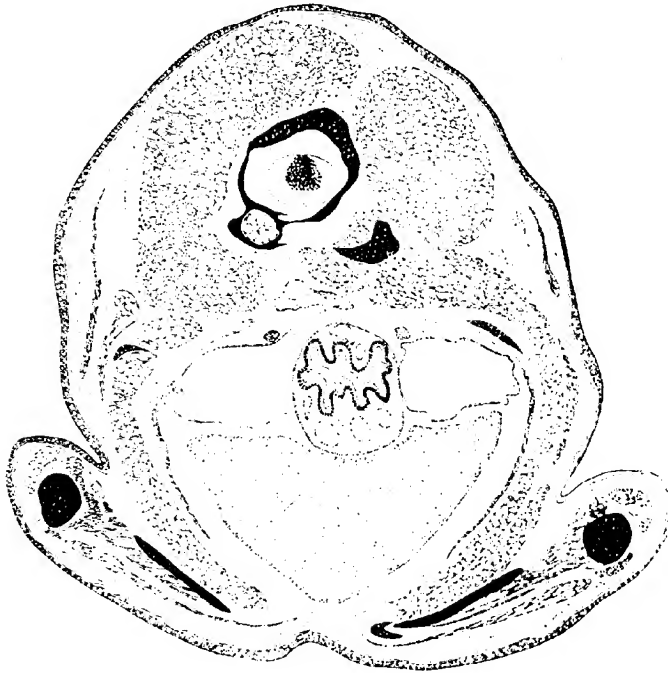


Fig. 13 Transverse section of scoliotic case ReSe 11 at the level of the fifth spinal nerve, showing right lateral displacement of the vertebral axis and asymmetrical development of the dorsal trunk musculature. Animal preserved 54 days after operation.  $\times 25$ .

4 is shown the extent of cellular differentiation within the cord through the limb level as well as that in the corresponding ganglia. The cellular ratios between the third and fifth nerve levels of the cord under the experimental conditions are seen to correspond closely with the normal.

*Cases ReSc 6 and 13.* Although the findings in these cases are not recorded as fully as those above described, the microscopic examination showed an adjustment on the part of the reversed limb level in all essentials the same as in the other cases. The

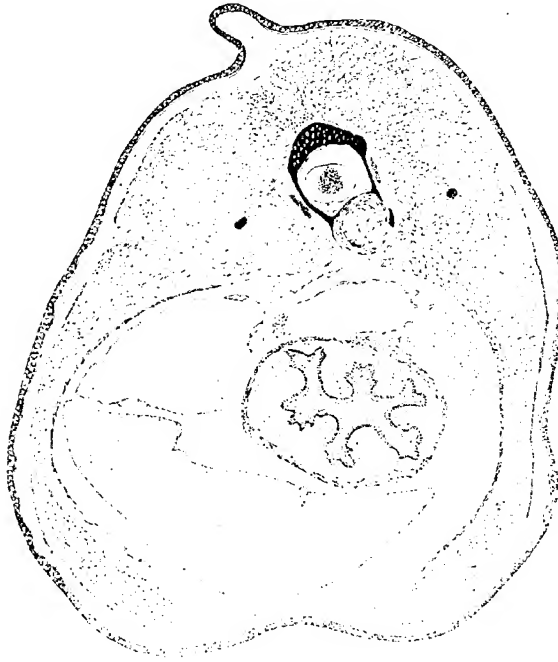


Fig. 14 Transverse section of scoliotic case ReSc 11 at the level of the ninth spinal nerve, showing left lateral displacement of the vertebral axis and asymmetrical development of the dorsal trunk musculature.  $\times 25$ .

relative size of the sections taken from the third, fourth and fifth nerve levels in case ReSc 6 are shown in figures 7, 8, and 9 (cf. figs. 1, 2, and 3). The brachial plexus in both cases was normal and the terminal branches supplied the muscles in normal manner (table 2). In these cases the swimming and limb reflexes de-

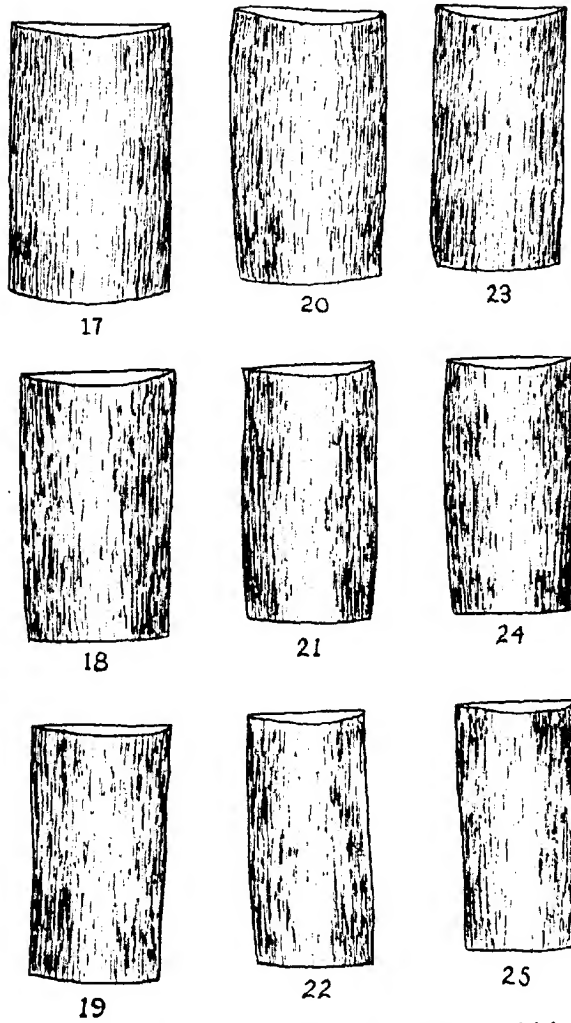


Fig. 15 Photograph of living specimen of *Amblystoma* larva (case ReSc 1) taken 53 days after operation. Anterior limb level of the spinal cord (third, fourth, and fifth segments) reversed.  $\times 2\frac{1}{2}$ .

Fig. 16. Photograph of living specimen of *Amblystoma* larva (case ReSc 6) taken 54 days after operation. Anterior limb level of the spinal cord (third, fourth, and fifth segments) reversed.  $\times 2\frac{1}{2}$ .

Figs. 17, 18, and 19 Drawings of reconstruction models of the left halves of the third, fourth and fifth spinal segments, respectively, of the cord (brachial segments) in a normal *Amblystoma* larva of 68 days (AS4).  $\times 65$ .

Figs. 20, 21, and 22 Drawings of reconstruction models of the left halves of the third, fourth and fifth spinal segments, respectively, of the cord (brachial segments) in operative case ReSc 1. Limb level of the cord (third, fourth, and fifth segments) reversed. Animal preserved 53 days after operation.  $\times 65$ .



Figs. 23, 24, and 25 Drawings of reconstruction models of the left halves of the third, fourth and fifth spinal segments, respectively, of the cord (brachial segments) in operative case ReSe 10. Limb level of the cord (third, fourth, and fifth segments) reversed. Animal preserved 53 days after operation.  $\times 65$ .

veloped normally. The larvae were fixed fifty-eight and fifty-four days, respectively, after the operation. Case ReSc 6 is shown in figure 16.

*ReSc 11.* This individual, which was operated upon when in stage 23, passed through the 'C-shaped' and 'S' reaction stages without any evidence of defects either externally or internally. At about the period when the limbs began to function (twenty days after operation) a slight scoliosis was noted. This condition became quite marked and remained so up until the preservation of the animal (fifty-four days after operation). Although this condition interfered with proper swimming, the limb reactions were normal.

Microscopic examination of this case showed a marked curvature of the vertebral column, the rostral portion of which was considerably deflected towards the right and the more caudal portion towards the left. In figures 13 and 14 are shown sections through the larva at the levels of the third and seventh segments, respectively. The spinal cord, although curving in correspondence with the vertebral column, showed no defects in development. There was complete union and the relative sizes of the various brachial segments correspond with the normal. The brachial plexus and the distribution of its branches were also normal (table 2). From the study of the nervous system in this animal it is seen that the defective posture was not the result of any defects in the nervous system, and the condition rather points to irreparable injury to the sclerotome during operative procedure.

#### DISCUSSION

In several series of foregoing experiments (Detwiler, '20 b, table 2, and '22, table 2) considerable evidence was brought forth which indicated a strikingly intimate developmental relationship between the anterior limb of *Amblystoma* and its normal nerves. This was suggested by the apparent propensity on the part of the normal brachial nerves to supply the musculature of the limb and shoulder when the latter were transplanted short distances (from one to three segments) caudal or rostral to their normal

site. A lack of specificity in this relationship was evidenced by those cases in which the limb was displaced at distances greater than the above mentioned, wherein it was found that other segmental nerves which normally supply an extraneous musculature became built up into plexuses for the supply of the limbs. It appeared significant, however, that caudally transplanted limbs, which did not receive their nerve supply from the normal limb region of the cord, were supplied from a higher level than that corresponding to the position occupied by the heterotopic limb. Of further significance was the observation that, when the normal limb rudiments were undisturbed and an additional anterior limb rudiment was transplanted a given number of segments caudal to the intact normal, the segmental nerves supplying this additional limb came from a level of the cord corresponding to the position occupied by the transplanted limb. This observation is in accord with those previously made by Braus ('05) and Harrison ('07) on Anuran forms.

Although in all these experiments it became obvious that the character of the intrinsic distribution of the nerves within the terminal systems (limb and shoulder) is dependent upon the primary mode of segregation of the developing structures within these systems and is thus the same in all cases, this observation suggested no clue as to the forces responsible for the establishment of initial connections.

From the results of the experiments reported in this paper, we have further evidence suggesting that the apparent tendency for the normal limb nerves to supply their proper end-organ, which was evident in the limb experiments, does not result from any inherent predisposition on the part of a given brachial nerve for its normal terminal musculature, a possibility which was originally considered. Under the conditions of the experiments, the reversed third and fifth segmental nerves take on respectively the functions of the original fifth and third, and in all cases the brachial plexus and its branches are of normal character (table 2) - thus showing that any relationship which does exist between the outgrowing limb nerves and their normal developing end-organ is in no way to be considered as representing any specificity between muscle and nerve.

Although in the previous limb experiments no explanation was given for the peculiarity of the nerve supply, the results did suggest, however, that the apparent 'preference' on the part of the limb nerves for their normal end systems when the latter occupied a heterotopic position, is no more than an exemplification of the same forces which bring about proper peripheral selectivity under normal conditions of development. Whether or not these forces are electro-chemical in nature as suggested by Kappers ('17, '21) in accounting for selectivity within the central nervous system, still remains a difficult problem.

In an analysis of the developing reflex mechanisms in Amblystoma as outlined by Herrick and Coghill ('15), Kappers ('21) suggested that initial contraction of the myotomes sets free action currents already present in the longitudinal fibers of the central nervous system, as was evidenced by the fact that the primary motor root fibers originate as collaterals from these longitudinal tracts (Herrick and Coghill, *op. cit.*, fig. 3), and that only later are the neuroblasts, which lie next to such tracts, activated to develop axones that replace these collaterals and form the real motor roots. Kappers (*op. cit.*) suggested the probability that in embryos, the proliferation of muscle has the same influence as functioning adult tissue and that this proliferating tissue may thus activate irradiations of the nervous currents from the spinal cord. Bok ('17) has shown that the connection between certain muscles and sometimes widely distant places of the central nervous system, has to be explained by the fact that the contraction of a muscle (the formation of which precedes the formation of the nerve roots) exerts a trophic action upon the central nerve fibers.

It may well be that in embryonic cellular proliferation certain conditions (electro-chemical?) are attained in different peripheral systems in such sequence as to attract axones from certain levels of the cord in preference to others in accordance with the synchronous bio-electric state existing in these levels. That the intimate developmental association, which was found to exist between the displaced limb rudiments and the nerves coming from the normal limb levels of the cord, may be an expression

of some such relationship seems not impossible. Certainly the character of connections in the present experiments are not explainable on mechanical grounds alone.

Another point of interest in connection with the spinal cord reversal experiments as outlined in this paper pertains to the stimuli involved in bringing about the normal extent of cellular differentiation in the various segments of the cord. Under normal conditions of development there occurs a gradually diminishing cellular proliferation as we proceed from the rostral to the caudal limits of the brachial level (third, fourth and fifth segments). An index of the extent of cellular proliferation is shown in table 3.

This gradient of differentiation, which characterizes the normal, has remained with remarkable constancy throughout various experimental conditions. First of all, it was noted that, when a limb rudiment was excised, the extent of cellular proliferation in the various levels on the limbless side of the cord was inappreciably affected by the absence of the limb (table 3)—thus suggesting that the extent of proliferation is not commensurate with the functional demands of the terminal musculature. Secondly, it was found that, when the limb level of the cord was excised and replaced by a more caudal unit of cord (seventh, eighth and ninth segments), the neuroblasts in the grafted segments of the cord undergo increased proliferation which is approximately equal in extent to that which characterizes the normal brachial enlargement (Detwiler, '23, tables 2 and 7). Thirdly, in the present reversal experiments, it is seen (table 3) that when the fifth segment is substituted for the third, cellular hyperplasia takes place in this segment so that the resultant degree of proliferation is approximately the same as in the normal third segment. Further, the third segment, which is interchanged with the fifth, fails to undergo its typical degree of development and attains only a measure of differentiation characteristic of the normal fifth.

From the constancy in the relative degree of differentiation within the various segments of the limb level under all the experimental conditions stated above, considerable evidence has

thus accumulated to suggest that the degree of proliferation which is reached in these levels of the cord is commensurate with the number of longitudinal conduction fibers normally terminating in these respective levels for the coördinate control of the appendicular reflexes. This would be in accord with Bok's ('15) stimulogenous fibrillation concept— a possibility which has been previously suggested. It would thus appear that a greater number of these longitudinal fibers must terminate at the rostral end of the anterior limb level than at the caudal end. According to this concept, there would occur increased irradiation of stimuli in a region where fibers from an activating bundle terminated, so that the extent of neuroblast differentiation in such a region would be activated in a ratio proportionate to the number of longitudinal fibers terminating there. That there are more brachial correlation fibers ending at the rostral than at the caudal limits of the limb level has been secondarily suggested by the behavior of limbs transplanted varying distances caudal to their normal site, since it was found that the coördinate function of such limbs gradually decreased as their segmental nerve supply became shifted towards the caudal limit of the normal brachial level and beyond (Detwiler, '20b, tables 1 and 2).

The question of the effect of reversal of the limb level of the cord upon the polarity of the intercalary neurones was not studied in these experiments. From the normal character of the developing reflexes in the majority of the cases, it is to be judged that internal readjustment is much the same, if not identical, with that described by Hooker ('17) for frog embryos, viz., a maintenance of anatomical polarity but a subsequent reversal of functional polarity.

#### SUMMARY

1. Reversing the anterior limb level (third, fourth and fifth segments) of the spinal cord in *Amblystoma* embryos has no effect upon the character of the developing responses to tactile stimulation other than that resulting from imperfect union in some cases. A greater percentage of normal results was

obtained when the operation was performed on embryos just after the complete fusion of the neural folds (stage 21 to 23) than when performed on embryos just prior to the period when initial reflexes begin (stage 29 to 30), table 1.

2. The developing limb reflexes and their coördinate movements are not affected by reversing the limb level of the cord. In all cases which survived (table 1), the limb reactions were normal.

3. Microscopic examination of sections of these larvae shows that the character of the brachial plexus and the intrinsic distribution of its branches are identical with the normal. The interchanged third and fifth segmental nerves carry on, respectively, the functions of the normal fifth and third. The character of peripheral connections under these experimental conditions strengthens a former interpretation, viz., that the apparent 'preference' which the normal brachial nerves show for the limb when the latter is transplanted into a heterotopic position (Detwiler, '22) is in no way an expression of any inherent specificity existing between muscle and nerve. They further corroborate previous observations (Braus, '05; Harrison, '07 and Detwiler, '20 b, '22); that regardless of the segmental innervation to the limb, the intrinsic distribution of nerves within the appendage is always the same and is evidently dependent upon the primary mode of arrangement of the other structures composing the limb.

4. The results of these experiments, when viewed in connection with previous limb experiments strengthen the view that initial connection of the outgrowing nerves with their normal terminal systems cannot be explained on mechanical grounds alone. It appears possible that proper peripheral selectivity may be determined by some such electrochemical mechanism as that suggested by Kappers ('17, '21) to account for selectivity within the central nervous system.

5. The extent of cellular proliferation within the various segments of the limb level of the cord in normal development is not maintained in the experimental animals with reversed cords (table 3). The reversed fifth segment undergoes cellular hyper-

plasia approximately equal in extent to that which characterizes the normal third segment, whereas in the reversed third segment, incomplete development follows and the degree of cellular differentiation approximates that which typifies the normal fifth segment (table 3).

6. The above observations as to cellular proliferation, when viewed in the light of the limb experiments and former spinal cord transplantation experiments suggest that the normal gradient of cellular differentiation within the anterior limb level of the cord is commensurate with the number of longitudinal correlation fibers normally ending in these various levels for the coördinate control of the limbs.

7. More evidence has accumulated in these experiments to indicate that the various segments of the cord are capable of remarkable readjustment and adaptation to experimental conditions.

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## ON THE LOCOMOTION OF THE LARVAE OF THE SLUG-MOTHS (COCHLIDIIDAE)

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The curious larvae of the slug-moths (Cochliidiidae, or Limacodidae) have an obvious resemblance to a naked mollusk, not alone in shape but also because their coloration recalls vividly that of the smaller exotic nudibranchs. It is not so well known, however, that these caterpillars demonstrate in their method of creeping an additional parallel to the gasteropods. Taking into account size, pigmentation, external projections of the body wall, stinging organs, and type of locomotion, one finds a surprising correspondence of these two so far-removed groups.

With chitons and with the majority of gasteropods pedal locomotion is accomplished<sup>1</sup> (Parker, '11; Olmsted, '17) by waves of neuro-muscular activity which rhythmically succeed one another upon the surface of the foot. In the pedal progression of sea-anemones (Parker, '17), likewise in the creeping of polyclads (Crozier, '18; Olmsted, '22), and in the locomotion of some holothurians (Parker, '21), an entirely analogous mechanism has been observed. Each 'wave' is constituted by a transverse area of the 'pedal' surface locally and momentarily withdrawn from contact with the substratum. Within this lifted zone the longitudinal contraction or extension responsible for progression is effected. These various instances of progression by the aid

<sup>1</sup> An unusual method of muscular progression, not involving the use of pedal waves, has been described in *Xenophora* (Crozier, '19) and more recently in *Strombus* (Parker, '22); these are snails in which the pedal surface of the animal is small in proportion to the mass of the body and shell, and the free surface of the foot is found in fact to act merely as an organ for temporary attachment of the body when it is extended at the beginning of a 'step,' the 'step' itself being executed by contraction of the body muscles.

of wave-like steps probably have it in common that each is referable to the activity of an organ in which a nerve-net, structurally interwoven in an intimate way with the tissues of a dermo-muscular wall, may be inferred to play an important part. In this respect the creeping of larval *Euclea* and other slug-caterpillars provides an instructive contrast.

Caterpillars commonly commence creeping by a forward movement of the anal pro-legs, initiating a peristaltic 'wave' of forward extension which runs to the anterior end. This movement may begin, however, at practically any level. It is seen to involve the whole of the tubular body-wall. Pro-legs and legs are the organs of adherence and of progression, their successive release and advancement being coincident with and determined by the worm-like peristalsis of the body. But the slug-larvae have the legs and pro-legs much reduced, the latter indeed practically absent; so that their method of crawling has commonly been described by stating that they appear to 'slide,' or glide upon the flattened, flexible, ventral surface, the latter being closely applied to the object upon which the larva is creeping (cf. Dyar and Morton, '95); the statement also occurs that the larvae use "the entire under surface in walking."

The exact method of progression cannot be made out unless the larva be examined from beneath. The ventrum is sufficiently adhesive to permit the creature's creeping upside down on a glass plate. This attitude is not 'unnatural,' for it is a usual one on the smooth-leaved trees (as apple, pear, cherry,) which these caterpillars frequent. Examined in this way it is seen that the ordinary creeping is by means of snail-like pedal waves, which because they run from posterior to anterior may be termed 'direct' waves (Vles, '07).

The full-grown larva of *Euclea indeterminata* (stages VII. VIII of Dyar, '97,) is about 1.7 cm. in length. The abdominal creeping organ, which may be called the 'foot,' measuring 1.1 cm. long by 2 mm. wide, is also the organ of adhesion. It is flattened and sharply marked off from the lateral areas of the body. The thoracic legs are puny, reduced, and are held curled away from the substratum. On the abdominal segments

'suckers' have been described, possibly homologous with the otherwise vanished pro-legs (Chapman, '94; cf. also Dyar, '98). Seven of these sucking pads are functional in the larva of *Euclea*. In the undisturbed caterpillar attached to a glass plate the paired elevations formed by these suckers are discernible on each of seven abdominal segments; the suckers are confluent at the midline, so that each segment shows a dumb-bell area not in contact with the glass. When the larva is disturbed by touching it, these 'sucker' areas disappear, the whole abdominal surface being pressed out in contact with the substratum. Since obvious suckers are lacking in some other genera of this family of larvae, I do not regard them as especially significant for attachment.

Adhesion is facilitated by the moist and sticky character of the surface of the 'foot.' To the surface of a leaf, or to glass, there must be pressed about one-third to one-half of the surface of the foot, in order that adhesion may be effected sufficient to support the weight of the animal; this minimal area for attachment includes at least three pairs of the 'suckers,' and is the relative proportion of the foot-surface required for attachment in other larvae lacking the suckers. The stickiness of the foot is also made plain by the fact that when a larva is creeping on glass the local retraction of the foot from this surface is distinctly audible. No one part of the foot is more adherent than the rest, and attachment to a new foreign surface may begin anywhere.

The locomotor waves occur one at a time upon the pedal surface; they are visible only on this surface, since they do not in any outwardly detectable way involve the lateral or dorsal musculature. In active creeping a new wave appears at the posterior end just as the preceding one is vanishing at the anterior limit of the abdomen. A wave is 1 mm. wide, and with each such step the larva advances 0.9 mm. The area of a wave is sharply elevated from the substratum, in some cases the depth of the wave being 1 mm. or a little less.

The 'foot' of the Sibine stimulæ caterpillar is similar to that of the preceding, but is morphologically more extensive, for it

comprises not only the abdominal, but also the thoracic ventral surface. The true legs are small and weak, and are usually kept retracted beneath the level of the foot; if the animal be turned over upon its four protecting dorsal protuberances, the legs may be seen somewhat extended. This larva is not quite so ready a creeper as that of *Euclea*. The surface of the foot, although not exhibiting segmental depressions, is adhesive to all common surfaces. The pedal waves are also similar in form, and in the fact that they appear one at a time. In both these species the ordinary pedal wave may begin at any abdominal level, just as is true of the peristalsis of ordinary caterpillars.

Similar observations were made with larvae of *Sisyrosea textula* and *Phobetron pithecium*; the last named is the most active creeper of the four studied.

When stimulated by a touch these larvae are not usually induced to creep, although a single pedal wave may appear. The chief utility of this wave seems to be that it 'irons out' the pedal surface into continuous contact with the substratum, thus giving the larva a firmer hold. The larva then gives a few slight jerky lateral swayings of the body, which clearly would be serviceable in driving home some of the numerous urticant spines with which the dorsal and lateral tubercles are thickly beset. Patient watching is necessary for opportunities to study the natural creeping act.

The average velocity of the pedal wave was found to be 3.8 mm. per second with *Euclea*, in *Sibine* 1.3 mm. per second, with *Sisyrosea* 1.42 mm. per second, and with *Phobetron* 1.6 mm. per second (at 23°C.). This velocity is less than that of the peristaltic body movements of ordinary caterpillars, for which the averages of a number of determinations upon some common species are as follows:

	cm. sec.
<i>Antomeris io</i> .....	2.0
<i>Diacrisia virginica</i> .....	12.0
<i>Aeronyx</i> sp.....	4.0
<i>Hemerocampa leucostigma</i> .....	1.3

In several unidentified caterpillars of smaller size speeds as low as 1.7 or even 1.0 cm. per second were observed, but still much

greater than that measured in the case of the pedal wave of the slug-caterpillars. The latter, in fact, corresponds almost exactly with the speed of the pedal wave in *Chiton tuberculatus* (Arey and Crozier, '19) and is therefore in this respect more comparable to the neuromuscular activity of the molluskan foot than to the peristalsis of the body in the earthworms or in other caterpillars. The point is of interest because it demonstrates the impossibility of distinguishing a condition of nerve-net conduction from one of conduction via a neuronic chain, on the basis of their speeds alone. The velocity of the 'pedal' wave in the caterpillars, although greater than that upon the basal disc of sea anemones (Parker, '17 a) agrees rather closely with that of the nerve-net conducted wave in *Leptoplana* (10 mm. per sec.; Crozier, '18), *Chiton* (2.2 mm. per sec.; Arey and Crozier, '19), *Helix* (1.3 mm. per sec.; Parker, '11), *Synaptula* (3 mm. per sec.; Olmsted, '17 b), for example, and even with the perhaps myogenic peristaltic waves upon the colonial body of the sea-pen *Renilla* (1.2 mm. per sec.; Parker, '20). Nevertheless there is every reason to suppose that a truly nerve chain conduction governs the progress of the pedal wave in the slug-caterpillars (cf. Crozier, '22 a, b).

In one particular the foot of these larvae differs from that of a *Chiton* or snail—the direction of the locomotor wave may be reversed. By stimulating the anterior end of a larva rather sharply, one or several waves may be started at this end, which will one at a time run posteriorly with about the speed of the usual direct wave or a little faster.<sup>2</sup> In *Chiton* reversal of creep-

<sup>2</sup> At the conclusion of the larval period these caterpillars form a thin brown cocoon within which they hibernate, without pupation, until spring. A true aestivation is probably evidenced in the fact that larvae removed from cocoons creep much more slowly than before. The speed of the direct pedal wave in the larvae of *Euclea* removed from cocoons after three to six days was 8.1 mm. per second, and of the retrograde wave 6.9 mm. per second. With *Sisyrosea* the normal velocity of the retrograde wave was 1.64 mm. per second, and in *Phobetrion* 3.43 mm. per second—in each case much higher than that of the direct wave. Reversal of the pedal wave may take place before the wave has run completely from one end of the body to the other, and this frequently is the fate of induced retrograde waves which have run half the length of the creeping surface.

ing is not accomplished by reversing the direction of the pedal wave.

Turning to one side is accomplished almost exactly as in a Chiton, the posterior end of the larva's body being swung to one side as the pedal wave is initiated while the anterior end is similarly swung to the other side when it is lifted by the termination of the wave. About six waves or more are necessary for a semicircular turn. In *Sisyrosea* there may be induced 'waves' of the usual type, but which in one movement run from one side of the foot to the other; these also are employed in turning.

The theory of locomotion by means of pedal waves requires (Parker, '11) that the surface of the wave itself move forward (or backward, in the case of reversed creeping) while this part of the pedal area is removed from contact with the substratum. This is very clearly the case in the slug-caterpillars. The action of the ventral muscle-bands is visible through the translucent skin (under the binocular microscope), and in forward creeping it can be seen that in the posterior part of the lifted wave lateral constriction is correlated with longitudinal extension in the immediately anterior zone. In backward creeping, the order is reversed. The peristaltic pedal wave is therefore to be regarded as a derivative of the general peristalsis of ordinary caterpillars, and as in the 'myenteric reflex' of the vertebrate intestine it implies reciprocal innervation.

#### SUMMARY

Caterpillars of four genera of the slug-moths, lacking pro-legs and having the thoracic legs much reduced, are found to creep by means of waves of muscular activity which course from posterior to anterior end of the flattened, adhesive, ventral surface. These direct, monotaxic waves are similar in many respects to those upon the pedal surfaces of actinians, platyhelminthes, chitons, and gasteropods, although the nervous structures involved in the locomotion of the caterpillars by this peculiarly modified peristalsis are probably quite different. Reversal of locomotion involves reversal of direction of the pedal wave, and is rather difficult to induce, despite the fact

that the velocity of the pedal wave when running from anterior to posterior is distinctly greater than that of the direct wave.

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## WEIGHT CHANGES AND OXYGEN CONSUMPTION DURING LONG EXPOSURE TO DILUTE ANESTHETICS

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FIVE FIGURES

### INTRODUCTION

In the course of an investigation of the factors concerned in determining head formation in pieces of *Planaria dorotocephala* regenerating in non-lethal solutions of certain anesthetics, it was necessary to record the oxygen consumption and weights of the pieces, both in dilute anesthetics and in water, at intervals over periods of time varying in different experiments from twenty-four hours to two weeks. For purposes of comparison of animals and pieces of different physiological condition, the results were calculated on the basis of the amount of oxygen consumed during a standard period of time, two hours, per milligram. The changes in oxygen consumption per two hours per milligram, in relation to weight changes, when compared with controls, show some differences during exposure to different anesthetics. The present paper constitutes a record of observations and data obtained in these experiments and briefly summarized elsewhere (Buchanan, '22 d), together with data obtained in subsequent work on intact animals starving under similar conditions, undertaken with the object of extending the investigation. This species is no longer available to the writer in numbers sufficient to permit further studies of this nature.

## THE PROBLEM

Although disagreement as to the nature of the action of anesthetics prevails, much evidence has accumulated to show that dilute concentrations of many anesthetics in some way stimulate the activity of organisms, the stimulation being manifested by increased motor activity, increase in rate of growth, increased irritability, and in other ways. (For instances of such observations see Czapek, '13, p. 143; R. S. Lillie, '15; Winterstein, '19, p. 17.) In fact, agreement is general that, while relatively high concentrations inhibit, low concentrations stimulate such processes, for a time at least. Considerable evidence has also been advanced to show that low concentrations of many anesthetics induce increases in respiratory rate in both plant and animal tissues. The evidence varies greatly in detail, however, and, so far as the writer has been able to find, the data do not contain evidence of changes in weight during the period of increased respiratory activity.

Elfving ('86) showed that atmospheres both of ether and of chloroform increased the total carbon dioxide production of *Salix* leaves over periods of fifteen to twenty-four hours. Similar concentrations had similar effects on *Pisum* seeds. His data do not show possible fluctuations during the period of exposure. He regards the increase of carbon dioxide output as not resulting from the oxidation of the ether and chloroform. Kosinski ('02) found that the carbon dioxide production of *Aspergillus niger* increased gradually for a number of hours in environments of 0.2 per cent and 0.25 per cent ether, but he gives no data on possible changes in the organism. Gerber ('02) found respiration in the *Banana* increased, over twenty-four hour periods, by ether vapor. Zaleski ('04) found that certain concentrations of ethyl alcohol increased the carbon dioxide production of the bulb of *Gladiolus*, but that it decreased during the later period of exposure. It was shown by Baer and Meyerstein ('10) that the formation of acetone by oxidation of oxybutyric acid in the perfused liver of the dog was increased by trichlorethyl alcohol and other substances. Their data show considerable variations; the

agents were applied for very brief periods, and the results probably represent initial effects only. They record that Lussana and Roli found that ether increased the carbon dioxide production of liver and muscle tissue, while chloroform and chloral hydrate depressed it.

Irving ('11) in a study of the effects of chloroform on the carbon dioxide production in barley shoots and laurel leaves, found that continuous treatment with a small quantity of chloroform vaporized in an air current induced an increase in carbon dioxide production which was maintained throughout the period of exposure (eighteen hours). Her experiments appear to have been very carefully controlled, but deal more particularly with the effects of stronger solutions. Vernon ('12) found that the respiration of the perfused kidney was increased by the addition of certain weak solutions of alcohol. Winterstein ('14) showed that respiration in the spinal cord of the frog was increased during alcohol narcosis. Both Vernon and Winterstein employed periods of exposure limited to several hours, and their data do not in any way show the possible effects of the increased respiratory rate upon the weight of living substance. Tashiro and Adams ('14) found that carbon dioxide production of the claw nerve of the Spider crab increased after short exposure to 0.4 per cent chloral hydrate. They record no change in weight. A fifty per cent gain in weight is noted after like exposure to a completely narcotizing solution (4 per cent). Irwin ('19) showed that high concentrations of ether increased the carbon dioxide production and oxygen consumption of the petals of *Salvia*. Her data probably represent only the initial effects of lethal solutions, since the maximum exposure was but thirty-five minutes and the concentrations employed were relatively strong. Medes and McClelland ('20) showed that the oxygen consumption and carbon dioxide production of *Elodea* was increased over twenty-four hour periods by 3 per cent alcohol, 1.5 per cent ether, 0.05 per cent chloroform, and 0.05 per cent chloretone. They record some diffusion of chlorides in these solutions, and also slight decreases in size of the chloroplastids. Recently Bodine ('22) found that small doses of certain anesthetics induced increased carbon diox-

ide production in grasshoppers, but notes that the higher rate was not maintained throughout the duration of exposure (eighty minutes).

This list is by no means complete but serves to show that increase in respiratory rate is a common result of the application of dilute solutions of many anesthetics to the most varied sorts of plant and animal tissues. The duration of the period of exposure to the anesthetics has been limited in most cases to twenty-four hours or less, and the possible effect of the increased respiratory activity on the weight of the tissue has not been intensively considered.

It is true that changes in weight do not always indicate changes in the mass of living substrate. Increase in weight by imbibition of water during early stages of embryos is a well-known phenomenon. Furthermore, Moore and Herdman ('14) showed that the live weight of a lobster actually increased during seven months' starvation. This is certainly not true of the weight during starvation in *Planaria dorotocephala*, as extensive work by Child ('19 a, and other papers) and Hyman ('19 b) has shown. The animals undergo progressive decrease in weight which is in general correlated with decrease in size, and imbibition of water is of minor importance as regards weight changes (see also Berninger, '11). The conditions, so far as weight is concerned, are essentially the same during the regeneration of pieces; as regeneration proceeds and the new individuals are reconstituted, the weight decreases (cf. Hyman, '19 c, pp. 70 to 75, col. 3). This decrease in weight in planaria during starvation or regeneration may be regarded as due, 1) to utilization of food in the alimentary tract during the early stages; 2) to utilization of reserves of materials in the tissues; 3) ultimately, to consumption of the protoplasm. The present paper presents data which are of interest in connection with the following questions: 1) Does the weight decrease more rapidly during the increase of respiratory rate induced by exposure to dilute anesthetics? 2) Are the conditions similar for several anesthetics? 3) If the exposure is continued, does the increased respiratory rate remain constant or does it continue to increase or tend to return toward the normal?

## MATERIALS AND METHODS

The experiments reported here are of two general types, *a*) those dealing with the oxygen consumption and decrease in weight of pieces of *Planaria dorotocephala* regenerating in dilute solutions of anesthetics, and, *b*) those dealing with the oxygen consumption and decrease in weight of intact animals under similar conditions. Since the results as regards changes in oxygen consumption and weight were of the same nature in both, they will be considered together. The following anesthetics were employed: ethyl alcohol, chloroform, chloral hydrate, and, to a lesser extent, chloretone and ether. Particular attention was given to the purity of the substances; the best grades obtainable were secured; the alcohol was double or triple distilled. The strength of the solution of each substance used was determined by the viability of the animals. The maximum concentrations of these agents in which the animals will live for prolonged periods, varies with the physiological condition of the animals; young animals are more susceptible than old, and pieces greatly stimulated by section are more susceptible than pieces less stimulated. The solutions used were, therefore, concentrations in which the most susceptible animals or pieces lived without external evidence of injury. In general, with the exception of mol.1 10 alcohol, these concentrations were non-narcotizing. Intact animals were slightly more active in the anesthetic for a day or more, but after that period there was no appreciable difference in the behavior of the experimental animals and their controls. In mol.1 10 alcohol the animals were partially anesthetized for several days, but gradually recovered.

The intact animals, fifty or more, or the pieces immediately after section, were placed in a 500-cc. Erlenmeyer flask filled with the appropriate solution of the anesthetic. At the same time a like number of animals, or of pieces, as nearly as possible of the same size and physiological condition as the first set, were placed in another flask and the flask filled with water. This second set served as the control. The manner of cutting the pieces and of obtaining data on oxygen consumption has been

described in some detail elsewhere (Buchanan, '22 d) and need not be redescribed here. The oxygen analyses were made by the Winkler method with slight modifications of the method as employed by Birge and Juday ('11). The titrations were carried out in the presence of the dilute anesthetics; however, any possible error thus introduced is of no importance, since it would occur in both the blank sample and that drawn from the flask containing the animals.

After the oxygen analyses were made, the animals or pieces were weighed. Accuracy in weighing is obviously of primary importance; however, weighing living planaria with accuracy and without injury presents a number of difficulties. Drying must be avoided, since partial drying and handling in the semi-dry state produce injuries that disturb the normal respiratory conditions. On the other hand, wet weighing introduces the error of weighing varying quantities of water. It is admitted that these difficulties were only partially avoided. The most satisfactory method was by pouring the animals or pieces into a filter funnel lined with hard surfaced filter paper, and, after the liquid had drained off, drawing the paper over a clean glass plate until it adhered firmly. The animals or pieces were then picked up with a thin spatula and weighed in a Paar weighing tube. The weighing was done as quickly as possible and the animals promptly returned to the flasks. It is impossible by this method to avoid weighing small quantities of water that adhere to the animals, but the results obtained were consistent and apparently the error introduced was fairly constant; two sets of animals of as nearly as possible the same size and physiological condition show very slight differences in rates of oxygen consumption per milligram.

After the initial determination of the oxygen consumption per milligram per two hours was made, the flasks were set aside in darkness; that containing the control animals being filled with water and the one containing the experimental animals filled with the solution of the anesthetic. Both were tightly stoppered; a small air space was left under the stoppers to allow for changes in volume with temperature changes. The solution and water were replaced by fresh every forty-eight hours; in some experi-

ments, every twenty-four hours. Throughout the duration of an experiment at intervals of several days determinations of weight and amount of oxygen consumed per two hours per milligram were made, with temperature and other conditions the same as in the initial determination.

A word is necessary here concerning the factor introduced by motor activity of the animals during these tests of oxygen consumption. This source of error was reduced to the minimum by subjecting the flasks containing the animals to a temperature slightly lower than that of the room and keeping them in darkness during the test. Under such conditions the animals remain quiet and change position very little during the course of several hours. The animals and pieces subjected to the anesthetics were not more active than those in water except during the first day or two, and differences in motor activity therefore do not account for their differences in oxygen consumption. Motor activity may, however, account in part for certain minor irregularities appearing in the data of a single set of animals during the course of an experiment, and for slightly higher rate of oxygen consumption in the anesthetics during the first day (except in the case of alcohol, pp. 335 and 342).

#### DATA

With certain exceptions, the data are given in the form of curves, and for convenience in contrasting weight changes with metabolic changes the curves of the data on weight are superimposed on those of the data on oxygen consumption. In the oxygen consumption curves the ordinates represent fractions of a cubic centimeter of oxygen consumed per milligram per two hours, the abscissae representing the intervals between determinations. In the weight curves the ordinates represent the weight in milligrams and the abscissae are identical with those of the oxygen consumption curves. The examples given are selected as typical of the results in each case. No exceptions occurred and the only appreciable variations appeared to depend on the size of the animals, small animals attaining a higher rate of oxygen consumption than the controls earlier than large ani-

mals. The number of experiments on the effects of chloretone and ether is not sufficient to warrant publication of data that may be regarded as typical; the results thus far obtained are summarized briefly. A more complete study was made of the animals starving in alcohol than in the other agents; two sets were carried for ten weeks and two for six weeks. The susceptibility of the alcohol animals to lethal solutions of KNC at the end of six weeks was also tested.

#### *Alcohol*

Figure 1 gives the history of the oxygen consumption of a set of animals starving for six weeks in mol.1/10 alcohol, curve A, and of a control, curve B. Curves A1 and B1 are the respective curves of the changes in weight. In all, sixteen experiments were carried out with alcohol, the duration of exposure ranging from twenty-four hours (two experiments) to ten weeks (two experiments). Without exception the results agree, for the corresponding periods, with those represented in figure 1.<sup>1</sup>

Child (15, and other papers) has shown that differences in rate of metabolism may be detected, under certain circumstances, by subjecting organisms to solutions of toxic substances, particularly KNC, in concentrations that are lethal, but so dilute that death changes require several hours before complete in all regions. In planaria the progress of death in such solutions can be easily followed. It has been found that the region in which the rate of metabolism is highest is the first to die, the death changes then successively involving other regions in order of rate. When the rates of death of two animals of different physiological condition are compared, the difference is a rough measure of their difference in metabolic rate. The reliability of this 'susceptibility method' as a rough measure of comparative rates of metabolism has been firmly established by comparison with direct quantitative

<sup>1</sup> It will be noted that the oxygen consumption of the control animals at the end of six weeks is much higher than at the beginning of the experiment. This increase of rate of metabolism in planaria after prolonged starvation has been denied by some investigators. The writer has found it occurring in every case in which the period of starvation was prolonged.

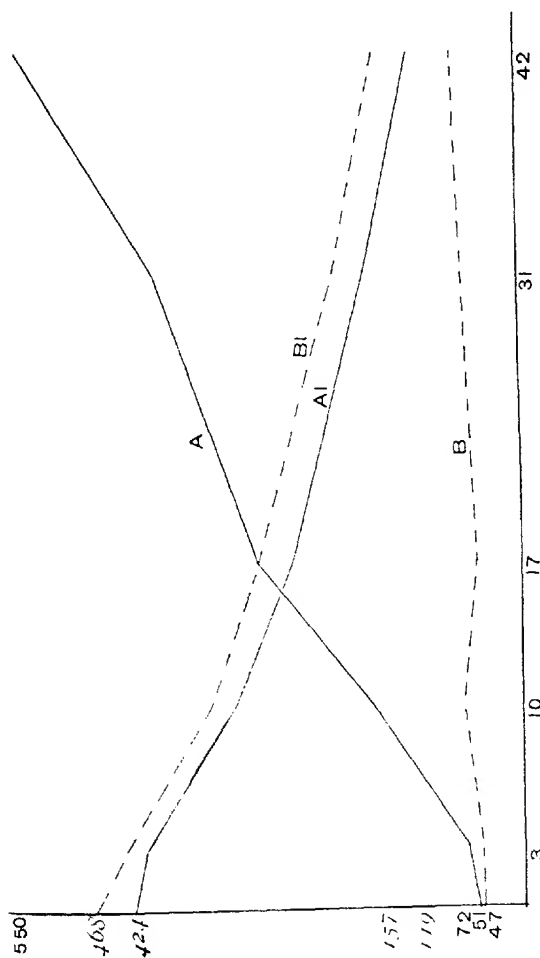


Fig. 1 A, oxygen consumption per two hours per mg. of 100 animals in mol. 1/10 alcohol for six weeks. B, oxygen consumption of control. AI, weight of alcohol animals; BI, weight of control. Animals 14 to 16 mm. in length and fed 72 hours before start of experiment. All tests conducted at 20°.

measurements of carbon dioxide production and oxygen consumption, by comparison with the progress of death due to lack of oxygen, and with other data.<sup>2</sup>

The susceptibility at the end of six weeks of four sets of ten each of the animals whose histories are given in curve A and curve A1 in figure 1 was tested and compared with that of the controls. The exact procedure in each test was as follows: Twenty animals of as nearly as possible the same size were selected with great care, ten from the alcohol stock and ten from the control, and placed in small Erlenmeyer flasks, the alcohol animals in one and the controls in another. The flasks were then filled with a fresh solution of KCN, mol.1 1000, and both flasks tightly stoppered. The flasks were then set aside under as nearly as possible the same conditions as regards light and temperature and at regular intervals the animals were examined with the aid of a hand lens and the progress of death in each noted. For convenience, Child has designated five stages in the progress of death and disintegration in planaria. In the present work, Child's criteria were followed and the number of animals in each stage was tabulated separately for each flask at every examination. Figure 2 is a graphic presentation of the results of all experiments, curves A, 1, 2, 3, and 4, being based on the data of the alcohol animals, and curves B, 1 and 2, on the data of the controls. In plotting curves of this sort, it is necessary to assign a numerical value to the proportion of the whole animal remaining alive at each stage of disintegration. Thus stage I is assigned the value 10, and since all the animals are in stage I at the start of the experiment, the value for each flask is 100 (plotted as ordinates). Stage II is assigned the value 8, and the combined value of the control animals at the end of two hours is reduced from 100 by the passing of some of the animals into stage II. In the alcohol animals the reduction from 100 is much greater, since more of the

<sup>2</sup> The discrepancy between Lund's results (21 a) on the effect of KCN on the rate of oxygen consumption of *Paramecium* and those of numerous investigators on the inhibitory effect of the cyanides on oxidations does not detract from the usefulness of the susceptibility method. On the contrary, it would seem that the apparent exception in the case of *Paramecium* demands further analysis.

animals have passed into stage II, and some even to stage III. The determinations for succeeding hours are plotted in the same manner. This method of plotting susceptibility curves is slightly modified from Child's method.

The striking difference between the curves of the alcohol animals and those of the controls requires no extended comment. Lack of material prevented further experiments of this sort, but

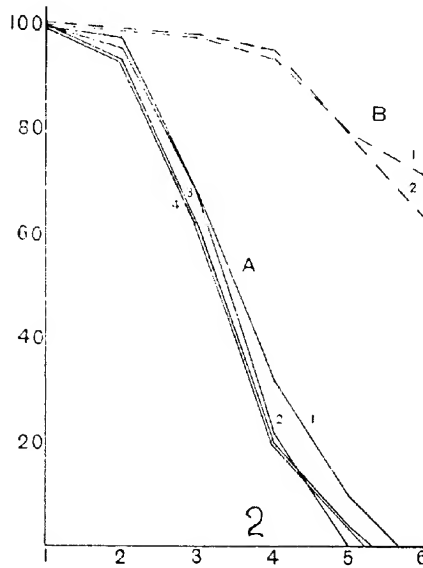


Fig. 2 Susceptibility of animals from figure 1, to mol/l 1000 KNC. A, 1, 2, 3, and 4, animals from alcohol set; B, 1 and 2 control animals.

in view of the very definite and almost identical results obtained in the four experiments tried, it is believed that further evidence of differences in susceptibility is not necessary. The results receive support in some work of Hunt ('07) who found that mice accustomed to alcohol feeding succumbed to methyl cyanide much more quickly than did control animals.

Two sets of larger animals with proper controls were carried for ten weeks with essentially the same results as in the examples given in figure 1, except that with the larger animals the increase in amount of oxygen consumed per two hours in the alcohol animals was not so rapid as in curve A, figure 1, the high point not being attained until the tenth week.

Figure 3 gives the record of a set of pieces regenerating in mol.1/10 alcohol for fourteen days, and of the control. It will be noted that the scale of the abscissae differs slightly from that in figure 1; however, the curves in figure 1 and figure 3 show the similarity in the results of the two experiments.

It has been found that when pieces are isolated from the body of planaria, a period of stimulation ensues which persists for twelve to twenty-four hours. This stimulation is marked by a distinct increase in oxygen consumption and carbon dioxide production of the pieces, by increased susceptibility to poisons, and by other evidences of increased metabolic rate (Child, '14, and other papers; Hyman, '19 c; Robbins and Child, '20). Indirect evidence indicates that in all probability this stimulation is of nervous origin except in the cells at and near the cut surfaces of the piece (Buchanan, '23). Strong solutions of chloroform, ether, chloral hydrate, and chloretone inhibit this stimulation and thus to an appreciable degree prevent the increase in oxygen consumption that normally follows section. It has been found possible by this method to control the regeneration of new individuals to some extent. The nature of the conditions controlling regeneration and their experimental control has been discussed elsewhere and will not be entered into here. From the results obtained in regeneration experiments and from the behavior of the animals there is clear evidence that mol.1/10 alcohol narcotizes the nervous and other transmission paths; however, the oxygen consumption of the pieces in this concentration of alcohol is higher immediately after section than in control pieces. The explanation offered for this difference between the effect of alcohol and that of strong solutions of other anesthetics is that the oxygen consumed is concerned in oxidizing the alcohol and that this source of oxygen absorption masks any inhibitory effect of the agent on the stimulation of

section (Buchanan, '22 d). As will be noted later (p. 349), this explanation seems well founded. It seems probable, therefore, that in the results given in figure 1 and figure 3 the oxygen consumption is general in the tissues and does not result from a purely nervous effect. The same is also probably true of the effects of the other anesthetics when employed in non-narcotizing concentrations.

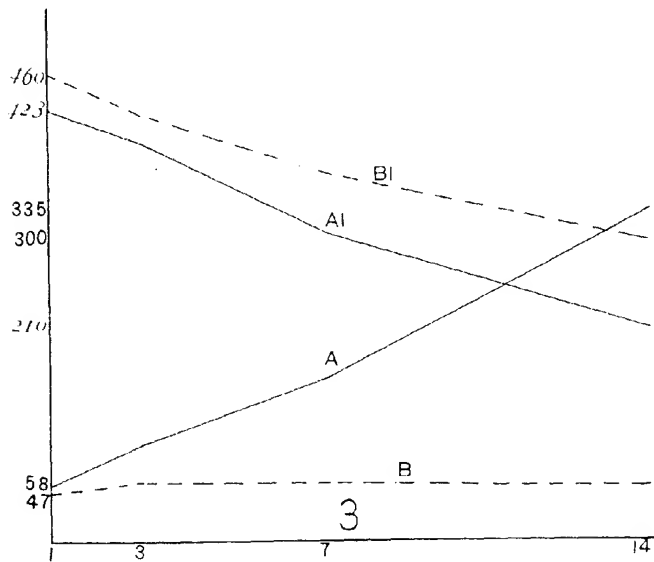


Fig. 3 A, oxygen consumption per two hours per mg. of pieces of *Planaria* regenerating in mol. 1-10 alcohol for two weeks; B, oxygen consumption of control pieces. A1, weight of pieces in alcohol; B1, weight of control pieces. All tests at 20°.

In figure 1 the curves A1 and B1 show that the weight of the animals decreases at practically the same rate in both alcohol and control sets. This is somewhat unexpected in the light of the fact that the oxygen consumption of the alcohol animals mounts above that of the control steadily and rapidly throughout the duration of the experiment, and also in view of the fact

that the greatly increased susceptibility to KNC suggests that the intrinsic metabolic processes involving oxidations are greatly stimulated. In figure 3 the weight of the pieces in alcohol decreases slightly more rapidly than that of the control. The results in other experiments are intermediate between these two examples or else like figure 1. The data justify the conclusion that the decrease in weight in alcohol when compared with controls is not related intimately to the increase in rate of oxygen consumption.

#### *Chloroform*

Figure 4 (plotted on the same scale as fig. 3) shows the history of the oxygen consumption of a set of pieces regenerating in mol. 1/3000 chloroform for eleven days, curve A, and of the control, curve B. Curves A1 and B1 are their respective weight curves. Comparison of figure 4 with the alcohol results in figure 1 and figure 3 shows that while at the end of eleven days the animals in the chloroform consume slightly more oxygen than their controls, the differences are much less than the differences between the alcohol animals and their controls at the corresponding period. It must be pointed out, however, that the data are not strictly comparable, for the solutions are not iso-molar. A study of iso-molar solutions of these agents is not practicable with planaria; the solubility of chloroform in water is so slight that it is not possible to make a mol.1/10 solution. Furthermore, even a mol.1.1000 solution of chloroform is so toxic that the animals are killed in less than forty-eight hours. On the other hand, mol.1.3000 alcohol (ca. 1/600 per cent) is so dilute that its effects cannot be detected with certainty.

Comparison of the weight curves of alcohol and chloroform shows that the decrease in chloroform in eleven days is much greater when compared with the control than in the case of the animals subjected to alcohol. This was very generally true in the experiments with chloroform, although the decrease in weight was not always as great as in the example given.

The work with chloroform includes fourteen experiments, eight with pieces and six with intact animals, the periods of

exposure ranging from three days to two weeks. In all experiments the results agree in substance with the example given in figure 4.

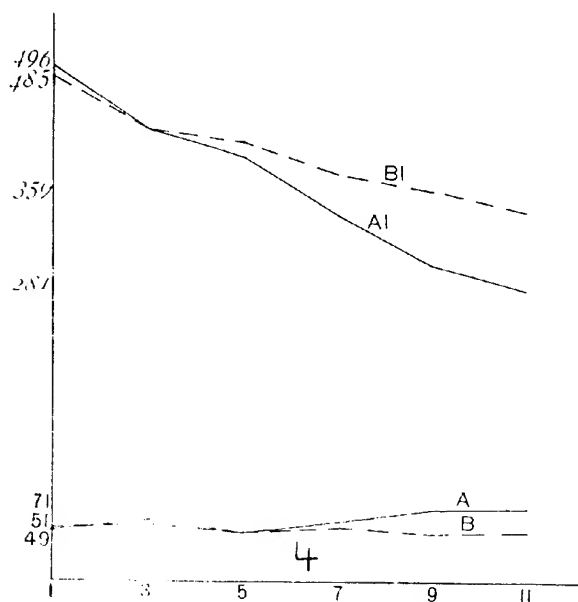


Fig. 4 A, oxygen consumption per two hours per mg. of pieces of *Planaria dorotocephala* regenerating in mol.1 3000 chloroform for 11 days; B, oxygen consumption of control. A1, weight of pieces in the chloroform; B1, weight of control. All tests at 21½°.

#### *Chloral hydrate*

Figure 5 shows the oxygen consumption results obtained in two weeks exposure of animals to mol.1 825 chloral hydrate, curve A, and the control, curve B, the weight curves being designated as A1 and B1, respectively. The data show that the increase in oxygen consumption in this concentration of chloral hydrate is greater than in M 3000 chloroform, but much less than in M/10 alcohol. The rate of oxygen consumption does not

begin to rise at once, as is the case in alcohol. For several days it remains the same or even slightly below that of the controls. Some time after the fourth day, however, the rate of oxygen consumption of the animals in chloral hydrate rises above that of the controls and continues to rise throughout the duration of the experiment. As regards decreases in weight, in most of the fourteen experiments with chloral hydrate the decrease in weight during the early periods of exposure was slightly less than that of the controls. Approximately coincident with the rise of the rate of

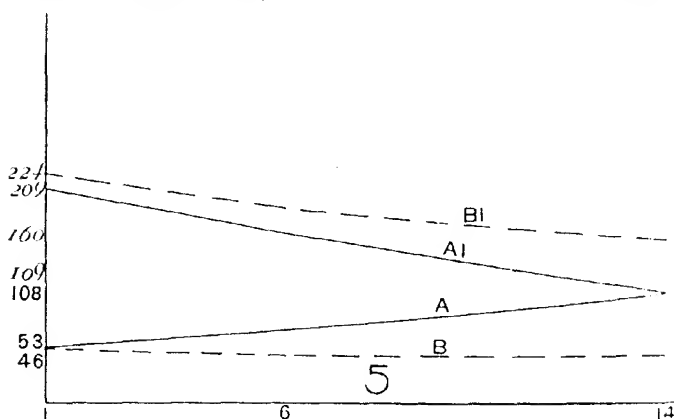


Fig. 5 A, oxygen consumption per two hours per mg. of 75 animals starving in mol.1 825 chloral hydrate for two weeks; B, oxygen consumption of control. A1, weight of animals in chloral hydrate; B1, weight of control. Animals fed 72 hours before start of experiment. All tests conducted at 18½°.

oxygen consumption above that of the controls (fourth to sixth day), the weight begins to decrease more rapidly so that at the end of ten to fourteen days the total decrease of the experimental animals was distinctly greater than that of the controls. The possibility of loss of substance due to disintegration is not excluded in these results. Highly susceptible animals, i.e., young animals or animals subjected to prolonged starvation, show some disintegration of the tissues of the head if the exposure to M 825 chloral hydrate is continued beyond ten days. This effect is more pronounced and appears sooner when the room temperature is high.

The disintegration is regarded as external evidence of a lethal effect which may be concerned to some extent in weight decreases in experiments in which there is no external evidence of disintegration. The weight decrease in chloroform probably also includes some slight loss of disintegration. Six experiments with pieces and eight with whole animals starving in M 825 chloral hydrate were carried out, the periods of exposure ranging from five days to two weeks. The results agree in general with the example given in figure 5. Weight changes are more distinct in the experiments with whole animals and the results necessitate the modification of a statement in an earlier paper (Buchanan, '22 d) that the weight decreases at approximately the same rate in both experimental and control series.

#### *Chloretone*

Six experiments with pieces regenerating in M 1000 chloretone for two weeks were performed. The study is by no means complete, but the results obtained may be briefly summarized: The oxygen consumption of the pieces in the chloretone rises above that of the control, the rise continuing from the third day to the fourteenth. However, in no case was the oxygen consumption at the end of the fourteenth day more than one and one-half times that of the control. The weight of the animals in the chloretone decreases more rapidly than that of the controls during the later periods of exposure.

#### *Ether*

Six experiments with pieces regenerating in M 300 ether for two weeks were performed. Although the processes of regeneration are greatly affected by this concentration, and stronger solutions are more or less lethal, the oxygen consumption of the pieces in the ether did not vary appreciably from that of the controls at any time, nor did the weight decrease more rapidly. These results are not regarded as evidence that ether may not produce effects similar to those of the other agents employed. The explanation of the non-occurrence of the increase in oxygen con-

sumption and of the lack of effect on weight is probably to be found in the concentration used; results similar to those obtained with the other agents might be expected in other concentrations of ether.

#### GENERAL CONSIDERATIONS

In view of the fact that the results given here are in some respects different from those obtained by others, a further word concerning the possibility of error seems advisable. A number of possible sources of error other than those discussed above (page 336) remain to be considered.

The striking increase in oxygen consumption of the animals starving in these solutions, especially in alcohol, may be due to the accumulation of the micro-organisms in the flasks and not to the respiration of the animals. The data on susceptibility reduce this possibility. Furthermore, the flasks were carefully washed out at each determination of oxygen consumption, while the animals were in the filter funnel during the process of weighing. At New Haven, and frequently at Chicago also, it was necessary to heat the water before using because of its supersaturation with oxygen. It was noted that heating the water and then cooling and aerating by shaking thoroughly made no difference in the results of the tests.

A second possibility is that the animals give off during exposure to anesthetics some iodine-absorbing substance, the output of which increases from day to day and which by uniting with free iodine renders the titration of the samples inaccurate and worthless. This possible source of error is rendered exceedingly remote by the following test. If some of the liquid is withdrawn from the flask after the sample has been taken, and shaken with air, its iodine-absorbing power is found to be reduced approximately to that of the stock solution when saturated with oxygen at the same temperature.

Veržar ('12) gives data showing that the oxygen consumption of certain tissues is to an appreciable extent dependent on the oxygen tension in the environment, while others, notably the salivary gland, are more completely independent of the oxygen

concentration. In the case of the kidney Verzar shows that low oxygen tension increases oxygen consumption. Lund ('21 b) has presented data which indicate the minimum concentration of oxygen necessary to maintain the normal rate of oxygen consumption of *Planaria agilis*. The possibility of a relation between oxygen concentration below a certain minimum, and oxygen consumption has been recognized in this and previous work, and every precaution taken to assure an adequate supply in the flasks at all times.

From the data and from consideration of possible sources of error it is reasonable to conclude that the increase of oxygen consumption of planaria in the presence of dilute anesthetics is continuous and cumulative throughout extended exposure. There appear to be but two possible factors involved: either the oxygen is utilized in oxidizing the substance or the normal respiratory metabolism of the protoplasm is increased, or both factors may be concerned. In the case of alcohol, the facts indicate that the major part of the excess oxygen absorbed is concerned in oxidation of the alcohol. Ethyl alcohol is easily oxidized and Pringsheim ('08) and others have shown that it is oxidized readily and rapidly in organisms. Cushny ('12) states that in the human body at least 95 per cent of the alcohol taken in is disposed of in this way.

The lack of correlation between the very striking increase in oxygen consumption of planaria and weight changes apparently indicates that the alcohol is oxidized by some mechanism which is not closely related to the intrinsic anabolic and catabolic reactions in the protoplasm. This would not accord with the conclusions of a number of investigators, that the oxidation of alcohol is substituted for the oxidation of carbohydrates and that the presence of alcohol thus tends to the accumulation or retention of these substances. Under such conditions, one would expect to find the weight of the alcohol animals decreasing less rapidly than that of the controls. The discrepancy may be only apparent, however. The very greatly increased susceptibility to KCN strongly indicates that the intrinsic oxidative metabolism is greatly stimulated. One would expect this cumulating increase

in oxidations to be more or less closely accompanied by an increasing requirement for oxidizable substances. Under such conditions, weight changes might not show any retention or accumulation of carbohydrates.

As regards the results with chloroform, it is probable that the relatively slight increase in oxygen consumption is largely due to stimulation of the normal respiratory exchange and that the oxygen absorbed is very little concerned in oxidizing the chloroform, since this substance is not readily broken down in organisms.<sup>3</sup> This probability is supported by the fact that the weight of the animals decreases with relative rapidity in chloroform. That it is possible for small quantities of certain substances to induce increases in oxygen consumption was shown by Warburg ('10), who found that dilute solutions of sodium hydroxide doubled the oxygen consumption of sea-urchin eggs even without entering. Warburg also found that ammonium hydroxide, which penetrated the cells, also produced slight increases in oxygen consumption when employed in dilute solutions.

The data on the effects of chloral hydrate indicate that this substance is oxidized to some extent by the organism, although the data are not as suggestive as in the case of alcohol. The decrease in weight in chloral hydrate indicates that the decreases in weight in alcohol and chloroform, particularly the latter, are not necessarily due to continuous dissolving out of lipoids and fats, since chloral hydrate, which has similar effects on weight, is only slightly soluble in these substances. The chloral hydrate data also show that an increase in oxygen consumption occurs in both water and lipoid soluble anesthetics when employed in dilute concentrations.

No examination was made of the waste products and carbon dioxide output. Undoubtedly such analyses would indicate more clearly the nature of the processes of metabolism affected by weak solutions of anesthetics. It may be added that close inspection

<sup>3</sup> Gwathmey ('18, p. 309) cites work by Thien and Fischer showing that chloroform may remain for twelve days after administration in the human body, a small fraction being broken down and the remainder excreted with expired air and in the urine.

of the animals in which the oxygen consumption was abnormally high—for instance, during the later period of extended exposure to mol.1/10 alcohol—failed to reveal any evidence of imbibition or of shrinkage.

#### SUMMARY

When individuals or pieces of *Planaria dorotocephala* are subjected continuously to mol.1/10 alcohol, the oxygen consumption of the animals increases much more rapidly than that of control animals from day to day. With animals of moderate size the oxygen consumption in alcohol at the end of six weeks is 700 per cent that of control animals in water. The susceptibility of the animals to lethal solutions of KCN also increases markedly.

The weight of the animals in the alcohol decreases slightly more rapidly than that of control animals (ca. 25 per cent).

Subjecting animals to mol.1/3000 chloroform for two weeks induces a slight increase in oxygen consumption. The weight of the animals decreases more rapidly than that of control animals (ca. 75 per cent).

Subjecting the animals to mol.1/825 chloral hydrate induces a rate of oxygen consumption that is distinctly higher than that of control animals. The weight of the animals in chloral hydrate decreases more rapidly than that of the control (ca. 40 per cent).

Incomplete work indicates that exposure to mol.1/1000 chloretone also induces an increase in oxygen consumption above that of control animals and also that their weight decreases more rapidly. Similar experiments with mol.1/300 ether failed to show any effect of this concentration on oxygen consumption and decrease in weight.

The probability of oxidation of these agents by the organism is discussed, together with the probability of stimulation of other oxidative processes in the presence of these agents.

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## A DESCRIPTION OF MATERIAL FROM A GYNANDRO- MORPH FOWL.

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ONE TEXT FIGURE AND THREE PLATES, TWENTY-ONE FIGURES

Through the courtesy of Dr. H. E. Schaef, a London physician, and Instructor in Anatomy in Western University Medical School, I obtained the body skeleton (entire except for a few of the cervical vertebrae, the head, feet and wing tips and the gonads) of a gynandromorph fowl, which he had had for some time in his poultry yard. The description and history of the bird as obtained from Doctor Schaef, is as follows. It appeared to be a hen, with neck feathering suggestive of the male, and tail feathers slightly longer than those of the normal hen. The comb and right wattle were typical of the cock, and it exhibited male sexual behavior. It attempted copulation with the hens with apparent success, but was less aggressive in this respect than the normal cock. It never was heard crowing, it did not have the strut of the rooster, and it did not fight other males.

The bird was suspected of laying small, normally shaped eggs, this assumption being based on the fact that the owner found such eggs from time to time, before it was killed and found none such afterwards. That this fowl was capable of laying eggs is shown by the developing ova in its ovary. Similar cases have been recorded. Boring and Pearl (18) cite the case of an abnormal cockerel, which constantly showed normal male sexual desire, but which laid 'twelve eggs and nested twice.' Eastman, quoted by these authors, describes a pullet that laid eggs, but that also mated with other pullets.

The bird was killed and prepared for the table. Doctor Schaef stated that as soon as the feathers were removed, it was evident

that the right side was much larger than the left. Doctor Schaefer eviscerated the fowl himself. Upon opening the abdomen, he found a testis on the right side, and on the left, an ovary and oviduct. The head and gonads were placed in formalin, and the skeleton preserved intact after the bird was roasted. This is the material which furnished the basis for the present paper.

#### DESCRIPTION OF GROSS MATERIAL

##### *Skeleton*

The most striking thing about the skeleton of this bird is that every bone on the right side is larger than the corresponding bone on the left. This is true also of those bones that are composed of fused halves of the right and left sides. The result is very profound malformation and distortion of the axial skeleton. There is present a most remarkable twisting and curving of the mid sagittal axis. If a surface be imagined joining the middle of the bodies of the vertebrae to the middle of the sternum, such a surface would show a marked convexity to the right both dorso-ventrally and antero-posteriorly. The whole pelvis is tilted so that the left side is lowered, while the right is much elevated. The mid line of the sacrum is curved with its convexity to the right. Because of the elevation of the right half of the pelvis, some of the greater length of the right leg is compensated for, but not all of it. In view of the great disparity in length of the right and left legs, it is astonishing that the owner noted no abnormality in gait.

The remarkable asymmetry of the skeleton is well shown in the photographs. Different views of it are given in figures 1, 2, 3, 5 and 6. Viewed from the dorsal surface (fig. 1), it is at once evident that the right side is larger than the left and that the mid line is curved. The capacity of the left side of the thorax is less than that of the right, due to the flattening of the ribs, and the decreased height of the thorax on the left side. Figure 2 displays the same asymmetry existing in the ventral aspect of the skeleton. Not only is the sternum as a whole displaced to the left because of the greater length of the ribs of the right side, but the line of

fusion of the right and left halves of the middle xyphoid process is very curved, its convexity being to the right. The right-sided preponderance is evident even in the processes of the vertebrae. This is seen in the free caudal vertebrae and the pygostyle in figures 1 and 2, and is even more marked in the hypapophyses of the cervical vertebrae, which are larger on the right side (fig. 5).

A view of the left side of the skeleton (fig. 3) shows the greater length of the right internal and external xyphoid processes, the greater height and length of the right half of the pelvis and the smaller size of the acetabulum, obturator fissure and ilio-sciatic foramen on the left side, when compared with the same structures in figure 6, which shows the right side. These latter differences are less than they appear in the original, since the reduction in figure 3 is a little less than figure 6.

Another view of the ventral surface of the skeleton is given in figure 5, so taken that the difference between the two coracoid processes is clearly brought out. The flatness of the left thorax and the deflection of the sternum to the left is well shown. Figure 6 of the right side of the skeleton shows some of the differences noted in the above description of the other figures.

The right and left sides of the head are shown in figures 8 and 9. Figure 7 is a photograph of the skin of the head after it was removed and spread out to show the size of the right and left wattles. The right measures 39 x 33 mm. and the left 29 x 27 mm. The comb, which is of the single variety, also differed on the two sides. Viewed from above, it showed in the posterior two-thirds a concavity to the right, and viewed from the front it showed dorso-ventrally another far more accentuated curvature, with its concavity on the right side. On the left the comb rose straight from the skin of the head. These foldings and curvings apparently took place to allow a single median structure to follow two different rates of growth, the effects of which are so evident in the paired structures of this bird. The beak was curved to the left (fig. 4), the mouth measuring 45 mm. on the right and 40 mm. on the left. The aperture for the eye and the nostril on the right each measure 1 mm. more than the corresponding structure on the left.

After the soft parts were dissected away from the skull (fig. 4) the right side appeared higher and larger than the left, the line of fusion of the two sides was curved toward the left, and the greatest diameter of the right orbit measured 4 mm. more than that on the left. The base of the skull, shown in figure 17, exhibits the line of fusion curved to the left, and the greater length and width of the right palate. Removal of the tongue and hyoid bone (fig. 18) reveals to a striking degree the asymmetry in this region, the tongue as a whole being deflected to the left, and its right side being greater in all its dimensions than the left. The right cornu of the hyoid is very much longer than the left. Even the fimbriae on the palate and tongue are larger on the right side.

Turning to the long bones (fig. 10) it is clear that the bones of both sides are normal in contour and proportions, but those of the left side are uniformly smaller than those of the right side. The left tibia, which cracked into several fragments due to its fragility after the roasting process, was both weighed and measured before it cracked. Figures 11 and 12 show the feet and wing tips which are in accord with the picture furnished by the rest of the skeleton. The spur on the right leg is larger than that on the left, although both are still small, the bird being young. In figure 14 the feet are spread out so as to show the greater length and thickness of the toes, and the larger spur and scales on the right side.

Comparisons of the weights and lengths of the bones are tabulated in table 1. This shows a striking uniformity in the ratio between the weights and lengths of the bones of the two sides. The average ratio showed that the bones of the left side were 66 per cent as heavy as those of the right. The lengths of the bones show a similar uniform proportion, only here the left side averages 85 per cent of the length of the right. Measurements of the bones of a normal hen of the same age as this gynandromorph S36-1 disclose no variation in the lengths of the two sides; also that the bones of the female half of S36-1 were normal in the extent of their development, since they practically coincided with those of the normal hen.

Measurements of the greatest transverse diameter of the right thorax was 45 mm., of the left only 30 mm.

TABLE 1

BONES	WEIGHT IN GRAMS				LENGTH IN MM.			
	Right	Left	Ratio	Left Right	Right	Left	Ratio	Left Right
Femur.....	19.5	12.2	0.63		120	103	0.86	
Tibia.....	24.5	15	0.61		169	138	0.82	
Foot*.....	66.5	42.7	0.64		220	190	0.86	
Humerus.....	9.3	6.1	0.66		105	90	0.86	
Radius.....	1.8	1.3	0.72		98	83	0.85	
Ulna.....	4.5	3.1	0.69		103	90	0.87	
End of wing*.....	29.3	13.2	0.65		97	80	0.82	
Scapula.....					102	91	0.89	
Coracoid.....					80	68	0.85	
Clavicle.....					93	75	0.80	
Internal xyphoid.....					88	79	0.89	
External xyphoid.....					33	29	0.88	
Middle xyphoid.....					120	102	0.85	
Pelvis.....					145	123	0.85	
Third thoracic rib.....					108	98	0.90	

\* The foot and the end of the wing did not have the skin or soft parts removed in either the weight or length estimations but were weighed and measured in the condition seen in figures 11 and 12.

TABLE 2

	LENGTH OF BONES	
	Gynandromorph + side	Normal hen of same age
	mm.	mm.
Humerus.....	90	88
Ulna.....	90	86
Radius.....	83	78
Femur.....	103	101
Tibia.....	138	143
Scapula.....	91	94
Coracoid.....	68	70
Clavicle.....	75	75
Pelvis.....	123	130
Middle xyphoid.....	102	110

Examination of the macroscopic appearance of the gonads (fig. 13) showed a normal looking testis weighing 10.86 grams, and measuring in the formalin-fixed state 50 x 23 x 14 mm. The ovary was more difficult to measure, since the mass was so irregular, but it measured approximately 20 x 14 x 10 mm. for the larger part, and 10 x 10 x 5 mm. for the smaller. Its weight was 1.78 grams. Six ova, broken free from the ovary, were present, two of which were sectioned. The largest ovum measured 15 x 13 x 12 mm. The smallest measured 5 x 5 x 5 mm. The irregular mass attached to and situated above and to the right of the testis proved, upon microscopic examination to be liver.

The brain was dissected out, but was rather poorly fixed, since the formalin had not penetrated well. Figures 15 and 16 give the dorsal and ventral aspect of the brain. The right cerebral hemisphere appears distinctly larger than the left; the optic lobes seem to be about equal. The right side of the medulla seems larger than the left.

Due to its marked curvature, the brain could not be cut accurately in half in order to weigh the two sides. It was sectioned and mounted serially, but due to the poor fixation was practically worthless for the study of tracts or cells. In order to determine the relative weights of the two sides, drawings were made on paper of uniform thickness of every other section throughout the brain by means of an Edinger projection apparatus, the magnification being five times. They were labelled 'right' and 'left,' also with the serial section number, then cut out, and the two sides weighed. The results were not as striking as the appearance in the gross would lead one to expect. The weight of the left hemisphere was 97.2 per cent of that of the right; the left optic lobe was 100.6 per cent of that of the right, while the combined weights of the left hemisphere and optic lobe were 98.3 per cent of the right hemisphere and optic lobe. Measurements of the greatest transverse diameter of the alternate sections of the hemispheres indicated that the left side measured 95.3 per cent of the width of the right, while the left optic lobe was practically the same as the right, being 99.3 per cent of the width of the latter. These figures show that the optic lobes were prac-

tically identical in size, while the right hemisphere was slightly larger than the left. In view of the constant relationship between the weights and the lengths of the two sides found in the bones, one would have expected to find a similar relation in the two sides of the brain. It is here, to a slight extent, but much less marked. Had the optic lobes shown the same ratio that existed in the bones, and the hemispheres the same ratio that they show now, one might have advanced the theory that the left side, being responsible for the motor control of the right side of the body, had developed larger cells and larger tracts than the right half, which was to control the smaller side of the body. Such a theory is untenable, since the optic lobes are equal in size, and the left hemisphere only a little smaller than the right.

#### MICROSCOPIC MATERIAL

*Testis.* A piece of testis was cut out and embedded in paraffin, and sections stained with Mallory's connective tissue stain, iron hematoxylin, hematoxylin and eosin and carbol fuchsin. The microscopic study shows it to be a perfectly normal testis, with the tubules well formed, the germinal epithelium showing numerous mitotic figures, and the lumen crowded with spermatozoa. Histologically, at least, it was a normal functioning testis (fig. 20).

*Ovary.* The ovary consists of a mass of ovarian tissue, through which is mixed more or less abnormal testicular tissue, according to the place from which the section is taken (fig. 21). Encapsulated in the center of the ovary is a large mass of adrenal tissue (fig. 21 a). The two types of cells of cortex and medulla are present, but not in their usual relationships, the groups of the two types of cells being more or less intermingled. Within the capsule are several groups of nerve cells of varying sizes, some cells being three times as large as their neighbors. In some sections the nerve cells are freely mixed in with the adrenal cells. Large nerves, with cells scattered through them, lie near the adrenal tissue. There are numerous blood vessels, much smooth muscle, and about the periphery in some sections numerous young oöcytes, and a moderate number of larger ones. In some places one finds a small amount

of nearly normal testicular tissue definitely encapsulated within a larger mass of testicular tissue in which the seminiferous tubules are mixed in with a large amount of ovarian stroma. Here the spermatocytes are in many cases separated from the basal cells, and are lying loose with an occasional sperm in the center of the tubule. In sections stained with Mallory's connective-tissue stain the 'interstitial cells' of Boring and Pearl, or the eosinophilic leucocytes, according to Goodale and Nonidez are numerous. Some follicles present are undergoing atresia; masses of luteal cells are present in the theca interna and in the corpora lutea.

#### DISCUSSION

Cases somewhat similar to the present one are the eight 'hermaphrodite birds' of Boring and Pearl (18); Hartman's fowl with functioning gonads of both sexes; the pheasant of Bateson, and many other cases too numerous to be recorded. These were all intersexuals, a condition exhibited by S36-1 on the left side; but it also belonged to the class of gynandromorphs in which are placed Boveri's bees, Bond's pheasant, and the birds of Poll, quoted by Bond. Lillie's free-martin has a great many points of resemblance to this case; in both instances the two sex hormones were circulating throughout a single individual, and so modifying the course of development.

With the exception of Bond's pheasant, which exhibited a bilateral asymmetry in the region of the tarsal bones and phalanges I have been unable to find any records of a case of complete bilateral asymmetry associated with a combination of secondary sex characters of both sexes. Monrad records eight cases of unilateral gigantism, four of which were right sided and four left sided; but they possessed no abnormality in the secondary sex characters. I recently saw a woman in a side-show who showed in a striking degree the phenomenon of bilateral asymmetry. She claimed to be the mother of one child, so that the generative tract was sufficiently female for reproduction, but she had the typical male voice, had to shave every day, and possessed the physique and features of the male. There was a well-developed breast on the left side and none on the right (she stated

that there never had been one on the right side); the whole left side, normal in its proportions, was uniformly smaller than the right. The left foot required a no. 7 shoe, while the right required a no. 9; the left leg was 5 inches shorter than the right; the left ulna 1 inch shorter than the right, and the fingers of the left hand, more tapering and smaller around than those of the right hand, also averaged  $\frac{1}{2}$  inch less in length than the right-hand fingers. I have no data as to the appearance of the external genitalia, but the somatic characters of the individual were modified on the right side especially, toward the male type, as shown by the greater growth of the skeleton, muscles and hair on the right side of the body and the absence of the breast on that side.

Much confusion has arisen through the wide divergence of opinion as to what constitutes the secondary sex characters in the domestic fowl. The conclusions of Boring and Pearl ('18) are at variance with those of Pezard, Morgan ('20 a) and Goodale ('16). This fowl, where conditions imposed by nature were ideal for this experiment, seems to bear out in practically every detail Goodale's ideas as to the relations between the sex hormones and the secondary sex characters. The theory that the secretion of the ovary inhibits the appearance of male plumage also receives support from this case. The luteal cells, held responsible for hen-feathering (Morgan and Boring; Morgan '20, b and c), are normally abundant in this fowl's ovary when compared with the ovary of the normal hen.

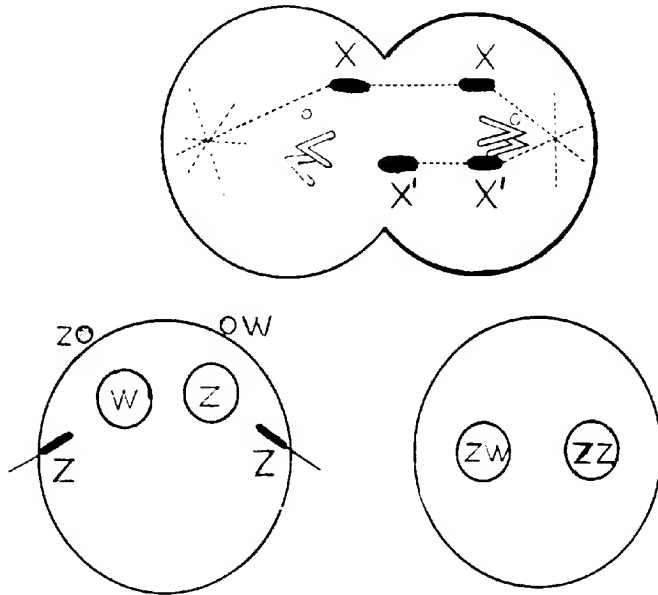
Examination of the testis does not support the theory that there are interstitial cells responsible for the male sex characters in the fowl. There was no atrophy of the seminiferous tubules, nor any evidence between them of cells usually designated as interstitial. Pezard's idea that the internal secretion of the testis is from the 'glandular parenchyma' seems more correct than that of Massaglia who holds that there is a true interstitial tissue.

Another cause of much discussion and one upon which some light is thrown by the present case is the supposed antagonism existing between the gonads. Moore ('21 a) found no such

antagonism, both sets of gonads growing well in the same animal, in which the somatic and psychical characters remained unaltered after the transplant unless the animal's own gonads had been removed previously ('21 b). Sand, using the same species as Moore had used, obtained results which he interpreted as indicative of antagonistic influences between the male and female gonads. Yatsu's experiments on parabiotic union in rats showed practically no inhibition of either gonad by the other. In the natural experiment of S36-1, both testis and ovary grew well and each functioned in the presence of the other.

The theories of sex hormones cannot fully explain the condition found in this fowl, for the hormones were equally accessible to both sides of the body. The bilateral asymmetry rests upon something more fundamental than sex hormones, namely the zygotic constitution of the individual. Two theories adapted from Morgan ('19) are here presented, which would explain the condition (text fig. A). The first postulates that in the first cleavage, one X chromosome lags behind and fails to be included in one of the cells. The X chromosome may disintegrate for some reason, in one of the cells, it being known from the work of Mayor that the X chromosomes are more vulnerable than the others. The side possessing only one X chromosome would be the female half, the hen being heterozygous for sex, and the other half would develop into the male. The second explanation is that the egg may possess two nuclei; each nucleus gives off its own polar body, and as the diagram shows, one gives off the Z, the other the W, chromosome. Two sperm then fertilize this egg, giving one side the full quota of male, the other of female, chromosomes. The same result would be obtained as in the latter case, if in the reduction division, two sperm of the many which penetrate the hen's ovum become functional instead of one; one of these fertilizes the egg, the other the polar body nucleus. Which of these is the true explanation must remain in doubt, since the material is lacking which would decide the question. That the two sides were different is shown conclusively by their different responses to the same environment.

There remains, however, the mass of abnormal testicular tissue embedded within the ovary, to be explained. Here we have a case very similar to that of the free martin described by Lillie. The male hormone of the right half of the body is circulating in the blood-vessels of the female half with the result that some seminiferous tubules are formed within the ovary. In cattle, the male hormone begins long before the female hor-



Text Figure A

mone is produced (Lillie and Bascom), so that the female tendency has little chance to express itself. Bond states, without giving his evidence, that the female hormone begins to function before the male, and ceases before the latter begins, in the case of the fowl. If this be true, one can explain the presence of so much normal ovarian tissue in this fowl.

Goldschmidt ('16, '17 a) assumes on the data from his experiments that sex is not only qualitative but quantitative. He

postulates that the constitution of the female is FFMm, and the male FFMM, where FF is greater than M but less than MM. FF is inherited maternally, being in the cytoplasm of the egg, and MM is a true mendelian factor, carried in the chromosomes. In different species, these factors have different values, so that he can obtain races where the value of M is greater than the value of FF in another race. Thus when crossed, the offspring, although zygotically females, are somatically males, the male substance being greater. He assigns values to these factors, stating, for example, that when  $FF - M > 20$ , or  $MM - FF > 20$ , the individuals are females or males respectively. If  $FF - M < 20$ , or  $MM - FF < 20$ , the individuals are female or male intersexuals. If values are assigned to these factors, one can see how the present case arose. Let F be 70, and M 100, then the female would have a balance of 40 in favor of femaleness, and the male would have a balance of 60 in favor of maleness, so that both would be somatically and zygotically of either the male or female sex. Since both these hormones were circulating throughout the whole body, the opposing factors were 4F and 3M, or 280 in favor of femaleness and 300 in favor of maleness. Since the male factor exceeds the female by 20, were this individual exactly comparable to Goldschmidt's moths, the whole individual would have developed as a normal male. The fowl is not a zygotic, but a hormonal intersexual (Goldschmidt '17 b); that is, its intersexuality is not determined at the moment of fertilization, but when the sex glands begin to produce their hormones. The female hormone, supposed to function first, influenced the development of the individual toward the female side; the male, coming into play later altered it in the direction of the male. Hence the ovary of the right side which normally degenerates, would meet only acceleration, and all the gonadal tissue of the right side would be male. On the left, the ovarian tissue which had already formed, persisted, but testicular tissue also became differentiated. It must be remembered in cases of hormonal intersexuals that the individual is not simply the algebraic sum of the two opposing factors. One hormone may produce an effect that the presence of the other cannot obliterate; the

effects of both hormones, somewhat modified, may be evident. A case in point is the hen feathering of this fowl. Although the male hormone was sufficient to produce sperm in an ovary, it could not produce cock-feathering, since in that respect, the female hormone predominates over the male.

That the hypophysis has a very close relation to growth is well known, but it is impossible to state what its condition was since the material was unfit for study. The secretions of any endocrine glands concerned with growth were equally available to the two sides, so that the explanation must rest upon the different genetic constitution of the two halves, and because of this difference they each responded to their appropriate secretion, and were less affected by the secretion of the opposite gonad. Apparently the soma upon which these hormones work, plays a large rôle in the result. In the free-martin, both sides zygotically the same, respond in an identical manner to the male hormone, but the result is an intersexual and not a real male. That the one sex cannot be completely transformed into the other in the case of the domestic fowl, and that the zygotic constitution of the individual largely determines the results obtained with exactly the same hormone is well illustrated by this case. The latter point may be a partial explanation of the varied results obtained by workers in this field.

I desire to express my thanks to Dr. H. E. Schaef, who gave me the material for this fowl, and who furnished the description and history of it.

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## PLATES

# PLATE 1

## EXPLANATION OF FIGURES

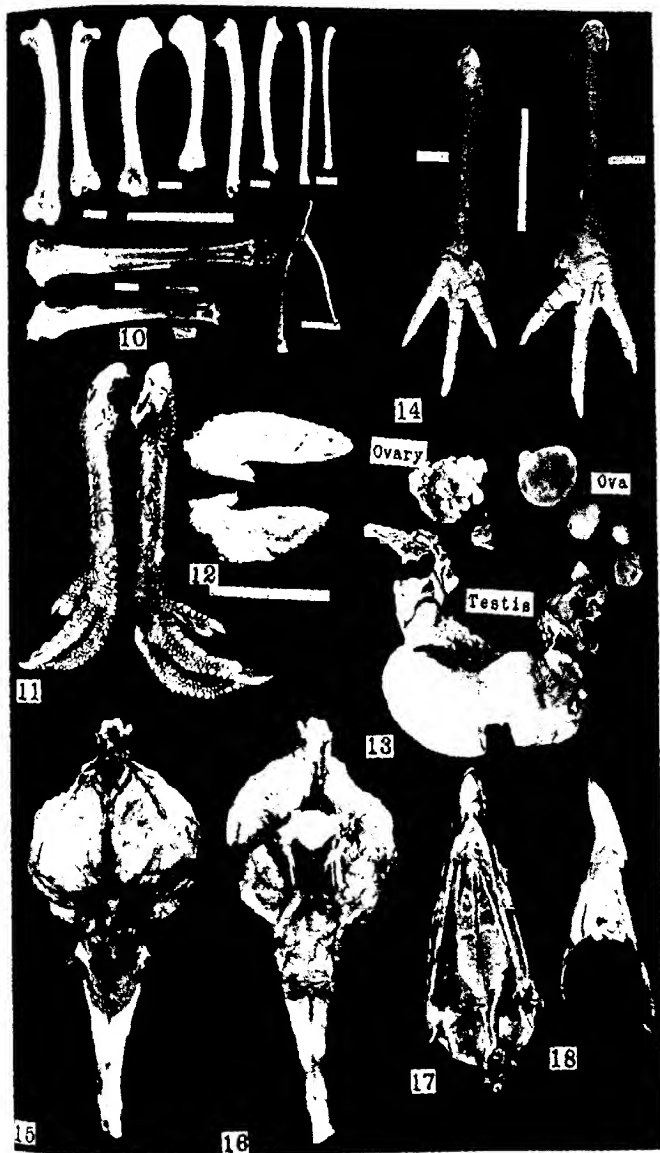
- 1 Dorsal aspect of skeleton of gynandromorph fowl. Right side male, left side female.  $\times 11$ .
- 2 Ventral aspect of skeleton.  $\times 11$ .
- 3 Left side of skeleton.  $\times 11$ .
- 4 Dorsal aspect of skull.  $\times 11$ .
- 5 Ventral aspect of skeleton, to show especially the coracoid processes and the ventral processes of the cervical vertebrae.  $\times 11$ .
- 6 Right side of skeleton.  $\times 12$ .
- 7 Skin of head spread out.  $\times 12$ .
- 8 Right side of head.  $\times 12$ .
- 9 Left side of head.  $\times 12$ .



PLATE 2

EXPLANATION OF FIGURES

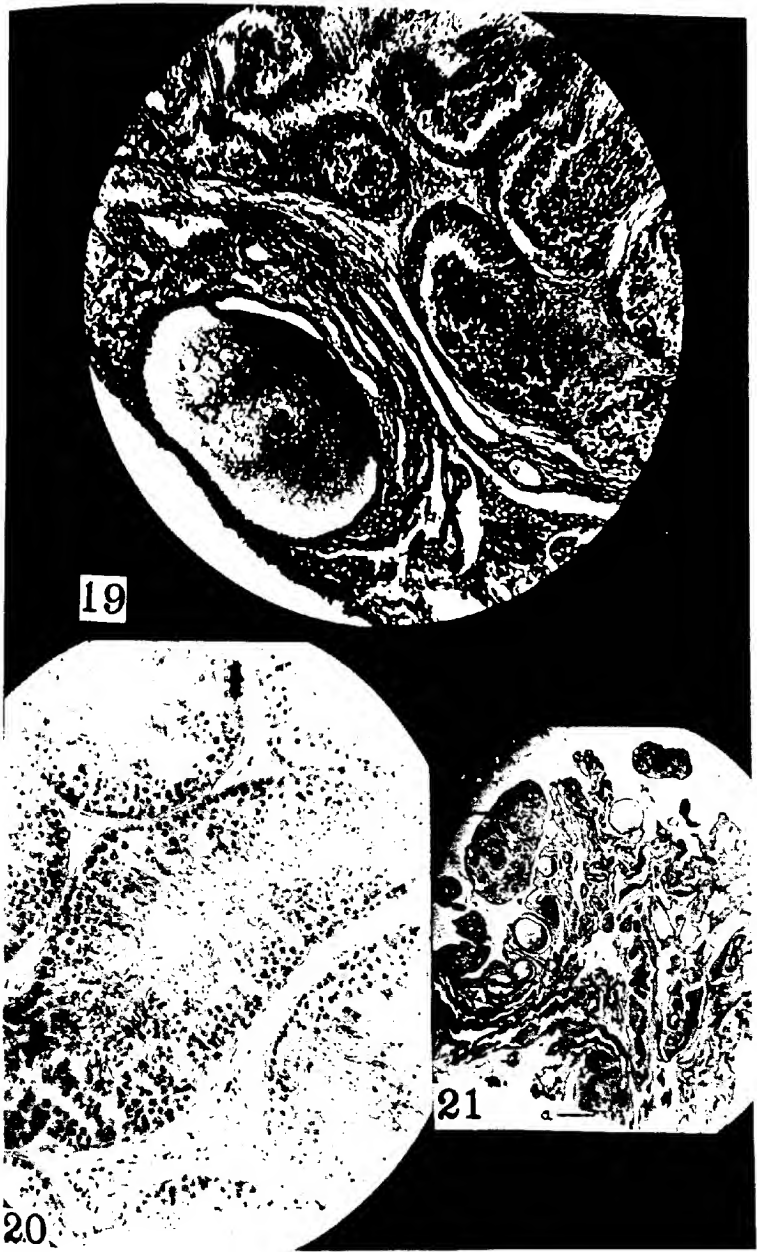
10. Long bones of skeleton. ( $\times 1/2$ .)
11. Feet. ( $\times 1/2$ .)
12. Distal part of wing. ( $\times 1/2$ .)
13. Gonads. ( $\times 1/2$ .)
14. Feet. ( $\times 1/2$ .)
15. Dorsal aspect of brain. ( $\times 1/2$ .)
16. Ventral aspect of brain. ( $\times 1/2$ .)
17. Palate. ( $\times 1/2$ .)
18. Tongue and hyoid bone. ( $\times 1/2$ .)



## PLATE 3

### EXPLANATION OF FIGURES

- 19. Portion of ovotestis from the ovary.  $\times 115$ .
- 20. Portion of testis of right side showing mitotic figures and spermatozoa.  $\times 200$ .
- 21. Section of ovary, showing oocytes, adrenal (a), and ovotestis (t), from which figure 19 was taken.  $\times 7$ .





## OBSERVATIONS ON AN ACQUIRED IMMUNITY TO A METAZOAN PARASITE<sup>1</sup>

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A few years ago Reuling first demonstrated that an animal may acquire immunity to a metazoan parasite. This was an important contribution to the literature of immunity and is of especial interest since this condition had been so long and variously sought without success. His results were gained upon fish acting as the host, and glochidia, the larval form of fresh-water mussels, as the parasite. It should be stated by way of explanation that the almost microscopic glochidium passes a parasitic period embedded in the superficial gill- or fin-tissues of an appropriate fish. Only under these conditions can metamorphosis to the free-living juvenile occur.

Reuling showed that at the first and second infections of fish a normal metamorphosis is completed in the average summer time of about two weeks. On the third and subsequent infections unusually heavy cysts were formed, but the glochidium sloughed off by the second or third day; this was accompanied by necrosis of the epithelium and a certain amount of glochidial disintegration. Hanging-drop preparations proved the blood of immune fish to exert a cytolytic effect on glochidia; tests indicated the probability of a weak precipitin in the antisera.

Having observed the general course of these experiments, I can testify to the accuracy of their broad conclusion. Nevertheless, incidental tests pursued during the last two summers bring out some additional information. My original objects

<sup>1</sup> Contribution no. 105. Published by permission of the United States Commissioner of Fisheries.

<sup>2</sup> Reuling, F. H. 1919. Acquired immunity to a animal parasite. Jour. Infect. Dis., vol. 24, pp. 337-346.

were two: first, to determine if more light infections are necessary to produce immunity than the heavy infections usually employed; that is, if there is an easy demonstrable quantitative factor; and secondly, to discover if an immunity once established remains effective through the following seasons. Both points have an important practical application in mussel propagation. Infections as they occur in nature are light in comparison with the heavy dosages given in propagative work. Should light infections prove as effective as heavy ones these feral fish might become immune and soon be useless as carriers. If an immunity builds cumulatively and holds from year to year it would further eliminate older fish from the host-function. These possibilities are especially significant in view of the extensive propagative program carried on by the Bureau of Fisheries. In restricted areas, rescued fish might thus be infected repeatedly without any certainty as to whether the glochidia metamorphose or slough.

In the experiments now to be described the host used was the large-mouth black bass, *Micropterus salmoides*; the glochidia were from the Lake Pepin mucket, *Lampsilis luteola*. These were the forms used largely by Reuling. A good artificial infection places about 2000 glochidia upon the gills of a fish.

First, my observations give some additional information on the number of infections necessary to produce immunity. Reuling found two infections sufficient for medium-sized bass, whereas large individuals may become immune after one period of parasitism and the small ones require three. This difference he explained on the basis of varied past experiences as hosts in nature—the older fish having had more opportunity to acquire partial immunity.

A lot of nearly three dozen black bass was infected repeatedly during the summer of 1922. The first two infections were uneventful. The third probably produced more or less immunity although there is only the positive record that the fish were free

For these fish I am indebted to Dr. A. D. Howard and Mr. Barry J. Austin, who also conducted the first infection both in 1921 and 1922.

of the glochidia on the tenth day, whereas a parallel infection on wild bass in another experiment required a week longer. At the fourth infection, the fish became practically clean after four days; all but a few were entirely free at six days and all on the seventh. There was no rigid correlation between large size and early immunity.

The results of the summer of 1921 are even more interesting. The first two infections were without especial feature. Two or three days after the third treatment the number attached gradually decreased. Yet the last fish was not entirely clean until the thirteenth day. At the fourth infection there was the same gradual reduction, some fish retaining glochidia until the third, fifth, seventh, ninth, and fifteenth days. At the fifth and last dosage conditions were similar, the fish becoming clean on the eleventh, nineteenth, and twenty-fifth days. This infection was early in September, but on the eight days following the water was as warm as the average for August. Two other fish were given a fifth infection on September 29th;<sup>4</sup> by this time the water temperature had lowered considerably and the fish retained the parasites, the sole survivor shedding metamorphosed larvae in the middle of the following May, when the water had again become warm. It is important to note that this fish lost many glochidia within the first four days, and still later the original number was reduced to one-half, yet despite this apparent partial immunity the glochidia remaining eventually metamorphosed.

These experiments indicate that immunity is not always acquired in a sharp, clean-cut fashion after the second or third infection, but that four, five, or more infections may be necessary, with a gradual building up of a semi-immunity. This also records for the first time as many as five infections on fish not yet immune.

Furthermore in these cases the shedding of encysted individuals may be progressive. Reuling found the glochidia were

<sup>4</sup> This was performed by Mr. Barry J. Anson, who kindly followed and recorded the later progress of both fifth infections.

sloughed in twenty-four to seventy-two hours and I have witnessed his experiments where this occurred. Yet in the two series described above, and in still another series having immunity not sharply defined, the shedding was prolonged over many days. Under these conditions the gill filament tends to become clean first along its proximal extent and considerably later at its tip. Thus, it is common to find such gills after a few days entirely free with the exception of cysts about their tips; these cysts may be thin and pedunculated.

In order to determine the relative efficiency of light and heavy infections in producing immunity, parallel tests were run. On the first infection both sets received an average dosage. Thereafter one lot was given three to four times the number of glochidia which the other received. The normally infected control showed signs of immunity on the third infection and at the fourth became raggedly immune, most being practically clean by the fourth day. In the lightly infected lot, on the second to fourth day of the fourth treatment there was shedding, and by the seventh day one-third were entirely free and the rest nearly so.

This experiment does not bring out any marked difference between the two groups but it seems to indicate a slightly earlier immunity by the fish which received the heavier infections. At any event there is not as much difference between heavy and relatively light dosages as might be expected. Possibly a still wider spread in the density of the infection, that is, heavy and extremely sparse, would be more effective. It appears, therefore, that the number of infections is more important as an immunizing factor than their heaviness.

At present it is impossible to state the permanency of an immunity once established. Of the 1921 fish none survived the hazards of winter and spring confinement—the last individual dying in May. Bacterial and fungus diseases also decimated the 1922 series which was to be wintered over in ponds. Nevertheless, scraps of information have been gained from the histories of individual fish and their behavior at infection which make it appear probable that the immunity is more or less permanent.

## SUMMARY

Immunity to glochidial parasites (e.g., *Lampsilis luteola*) may be acquired by fish (e.g., black bass) in two to five or more infections.

Fish that become thoroughly immune at the second or third infection slough the attached glochidia rather promptly within forty-eight to seventy-two hours.

Fish that require four or more infections acquire an ill-defined immunity, and glochidia are lost progressively over several to many days.

Light infections are practically as effective as heavy dosages in producing immunity, although there is apparently a quantitative difference when the spread is extreme. The number of infections seems to be more important than the degree.

The permanency of acquired immunity remains to be proved, yet there are miscellaneous records which indicate that it lasts at least one year.



## THE EFFECT OF FOOD ON LONGEVITY AND REPRODUCTION IN FLIES

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### INTRODUCTION

In 1921, while performing some experiments on the effect of various types of bacteria and protozoa on adult *Musca domestica*, *Stomoxys calcitrans* and *Lyperosia irritans*, the writer noticed that a close correlation existed between various types of food on the one hand and longevity and reproduction on the other. In common with animals, flies have special food requirements which are biologically interwoven with such factors as health, longevity, reproduction, etc. Growth cannot be inserted into these physiological expressions of food values, as is the case with rats (Osborne and Mendel), for a fly does not grow. It emerges from the pupal stage as an adult at which time its food requirements are absolutely different from the maggot food requirements. One can hardly imagine a greater change in diet than exists between that of the maggot and its adult fly stage. Perhaps, fundamentally, the difference is not so great, but superficially it seems enormous.

Some work has been done on the food requirements of maggots notably by Bogdanow, Guyénot, Wollman, Loeb, Northrup and Baumberger. Much is known in regard to the attractiveness of various types of foods to adult flies. Howard, Nicoll, Graham-Smith, Barrows, Hewitt, Richardson, Bishopp and others have given this subject much attention, and the entire matter of poisonous fly baits and traps is intimately associated with their labors. No one, however, with the exception of Guyénot, has given the subject of the food requirements of adult flies any serious attention.

Since the food requirements of adult flies was forcibly impressed upon the writer during the course of his experiments in 1921, he concentrated on this subject in 1922 and obtained data for the house-fly (*Musca domestica*), the biting stable fly (*Stomoxys calcitrans*), and the cattle horn fly (*Lyperosia irritans*).

#### METHODS

The food experiments were performed continuously from the first of May until the first of November. *Musca domestica* and *Stomoxys calcitrans* were reared in horse manure, and *Lyperosia irritans* in cow dung. As soon as the adults emerged they were removed and slightly etherized, so that the sex of each could be determined and males and females segregated. The males and females, necessary for a particular experiment, were then placed together in a sterile six-ounce, wide-mouth bottle. Cotton gauze tops held in place by rubber bands kept the flies from escaping. The flies were fed by permitting the liquid or semi-liquid food to drop upon the gauze top from a pipette. Three drops from an ordinary, sterile pipette are usually sufficient for from six to twelve flies. The third drop usually falls through, which is a good thing, since two or three individuals in a bottle always seem to prefer to take their food at the bottom instead of at the gauze top. Feeding, engorgement with the consequent tremendous swelling of the abdomen, and the passage of feces later are all indications that the food offered has been accepted. All food was prepared under sterile conditions and was stored on ice. Before feeding the food was warmed to 37° or 38°C. Experience has shown that it is necessary to feed flies daily without exception. No advantage is gained by feeding *M. domestica* or *Stomoxys* more than once a day, but *Lyperosia irritans* must be provided with fresh sterile blood twice daily, in the morning and in the evening. Unless this rule is adhered to rigidly the worker will obtain an unduly high starvation mortality which will seriously interfere with his experiments.

As a matter of routine, the flies were transferred to fresh sterile bottles three times a week and fresh tops replaced the old ones. However, if a particular bottle became unduly soiled, the flies

were transferred to a fresh bottle as soon as the condition was noticed. It is important to watch the bottles, because flies become soiled easily with liquid or semi-liquid food, especially when old and weak. When in this condition they frequently stick to the glass where they die of hunger or exhaustion. When found in this predicament and still alive, they can be saved by removing them with a pair of blunt forceps and washing them off under a gentle stream of water from the tap. In most cases flies so treated will recover and appear normal the next day.

All bottles were examined twice daily (morning and evening) for dead flies and all such were immediately removed. At this time the living and dead were counted and recorded. A note was also made of the eggs if any were present.

#### EXPERIMENTS WITH *M. DOMESTICA*

Tables 1 and 2 (pages 386-9) represent the results obtained with the adults of the house-fly. The tables are self-explanatory with the exception of a few matters that need further elucidation.<sup>1</sup> The different food tests were generally not performed at the same time, for as stated previously the entire work extended from May to November. Also, the tests with one type of food were not performed together. For instance, in cases where only two tests were recorded on the table one may have been made in June and the other in September. Where a large series of tests are recorded the experiments were begun at intervals throughout the season. This removes the possible criticism that here and there I may have dealt with a weak or a diseased breed of flies, for many of the experiments of a similar kind were performed with separate breeds. In a large series it was often necessary to make more than one test with the same breed, but this was never done with a small series involving only two or three trials. The flies used were all of normal size and well formed, showing that the larval food had been sufficient.

In the experiments given in tables 1 and 2, the bottles which contained mixed sexes held three males and three females. In those experiments in which only one sex is recorded either six

<sup>1</sup> General matters discussed apply to all tables (1 to 6.).

TABLE 1  
*Food experiments with M. domestica. 3 ♂ and 3 ♀ in mixed sex tests. In tests of one sex either 6 ♂ or 6 ♀*

EXPERIMENT	FOOD	NUMBER DEAD AFTER DAYS						OVIPOSITIONS AFTER — DAYS				
		1 dead	2 dead	3 dead	4 dead	5 dead	6 dead	First ovi- position	Second ovi- posi- tion	Third ovi- posi- tion	Fourth ovi- posi- tion	Fifth ovi- posi- tion
I ♂ ♀	None	1	1	1	1	1	2					
II ♂ ♀	None	1	1	1	1	2	2					
III ♂	Bouillon	4	4	4	4	4	4					
IV ♀	Bouillon	4	4	4	4	4	4					
V ♂ ♀	Bouillon	4	4	4	4	4	4					
VI ♂ ♀	Bouillon	3	3	3	3	3	3					
VII ♂	Horse blood serum	3	3	3	3	3	4					
VIII ♀	Horse blood serum	3	3	3	4	4	8					
IX ♀	Horse blood serum	4	4	4	4	4	4					
X ♂ ♀	Horse blood serum	1	3	3	3	3	3					



TABLE 2  
*Food experiments with M. domestica. 3 ♂ and 3 ♀ in mixed sex tests. In tests of one sex either 6 ♂ or 6 ♀*

EXPERIMENT	FOOD	NUMBER DEAD AFTER DAYS						OVIPOSITIONS AFTER—DAYS				
		1 dead	2 dead	3 dead	4 dead	5 dead	6 dead	First ovi- position	Second ovi- position	Third ovi- posi- tion	Fourth ovi- posi- tion	Fifth ovi- posi- tion
XXVIII ♂	Lump sucrose + bouillon	12	12	13	14	31	31					
XXIX ♀	Lump sucrose + bouillon	20	20	20	33	40	52	40 (8 eggs)				
XX ♀	Lump sucrose + bouillon	19	19	21	29	38	57	16 (few)	36 (few)			
XXI ♀	Lump sucrose + bouillon	14	16	22	23	23	33	16				
XXII ♀	Lump sucrose + bouillon	15	17	22	28	39	47	15				
XXIII ♂	Lump sucrose + bouillon	17 ♀	26 ♂	46 ♂	46 ♀	49 ♂	49 ♀	17				
XXIV ♂	Lump sucrose + bouillon	17	22	23	24	31	37	13	16	21 (few)		
XXV ♂	Lump sucrose + bouillon	13 ♀	16 ♂	20 ♀	20 ♂	30 ♀	35 ♀	12	15	20	24	
XXVI ♂	Lump sucrose + bouillon	8 ♂	14 ♀	22 ♂	31 ♀	32 ♀	33 ♀	11	13	18 (few)	25 (few)	33 (few)
XXVII ♀	Glucose + bouillon	12	12	12	12	25	29	18	23			
XXVIII ♂	Glucose + bouillon	6 ♂	6 ♂	7 ♂	15 ♀	45 ♀	49 ♀	19	24 (3 eggs)	33	43	45

		14	15	16	18	18	26	13		19	24 (few)	36 (few)
XXIX ♂ ♀	Lump sucrose + horse blood serum											
XXX ♂ ♀	Lump sucrose + horse blood serum	11 ♂	13 ♂	30 ♀	32 ♂	36 ♀	44 ♀	11				
XXXI ♂ ♀	Glucose + horse blood serum	3	4	5	14	15	18					
XXXII ♂ ♀	Glucose + horse blood serum	7 ♂	11 ♂	11 ♀	11 ♀	26 ♂	29 ♀	16		25		
XXXIII ♂ ♀	Soluble starch + bouillon	8 ♂	8 ♀	9 ♂	30 ♀	34 ♀	54 ♂	24 (3 eggs)				
XXXIV ♂ ♀	Soluble starch + bouillon	7 ♂	12 ♀	14 ♀	14 ♀	16 ♂	20 ♀	15				
XXXV ♂ ♀	Insoluble starch + bouillon*	3 ♂	5 ♀	6 ♂	9 ♀	11 ♀	18 ♀	13 (4 eggs)		14		

\* Heated to a semi-liquid cloudy paste.

males or six females were used. The second vertical column gives the type of food used. In the first two experiments no food was given to the flies. In the following four experiments bouillon was given. This bouillon, used in much of the work, came from the same laboratory lot. It was made from veal muscle and had a pH of 7.7. The lot contained 0.5 per cent NaCl and 1 per cent peptone. The bouillon was not fermented, but no sugar was added and it therefore contained only a trace of dextrose.

In experiments VII to X, inclusive, clear horse-blood serum was used. This was pipetted off from fresh, defibrinated, centrifuged blood and stored on ice.

In experiments XI and XII, the egg white from a hen's egg was removed under sterile conditions, diluted one-half with water, stirred and stored on ice.

In experiment XIII, 2 grams of commercial insoluble potato starch were added to 10 cc. of bouillon. This food was shaken each time prior to warming and feeding, the starch remaining in suspension.

In experiments XIV and XV, a piece of lump sugar was simply placed in the bottle and left there during the course of the experiments. In experiments XVI and XVII, the lump sugar was supplemented daily with three drops of distilled water.

Experiments XVIII to XXVI, inclusive, table 2, show that the lump sugar was supplemented daily with three drops of bouillon.

In experiments XXVII and XXVIII, the flies were fed with three drops of a solution made up by adding 1 cc. of a strong glucose solution to 10 cc. of bouillon.

In experiments XXIX and XXX, lump sugar was supplemented with horse-blood serum and in XXXI and XXXII, serum was used in conjunction with glucose at the rate of 1 cc. of strong glucose solution to 10 cc. of defibrinated horse-blood serum.

In experiments XXXIII and XXXIV, soluble corn starch plus bouillon was used. A 2 per cent semi-liquid starch paste was made by adding 2 grams of soluble starch to 100 cc. of bouillon. Some of the bouillon was first brought to a boil, after which a

suspension of starch in another portion of the bouillon was added to the boiling portion. This was stirred, brought to a boil again and removed. On cooling, the mixture was transparent and clear in color. Since the soluble starch is prepared from the insoluble form by the action of weak acid, a considerable amount of dextrin may have been present in this food. Tests seemed to confirm this belief.

In the last experiment, XXXV, the insoluble potato starch and bouillon were again used. In contradistinction to experiment XIII, however, the mixture was heated until a thick, semi-liquid, cloudy paste was produced.

In the columns 1 to 6, are inserted the number of days it took one, two, three, four, five, and six flies to die respectively. In these columns also the proportion of the sexes is given when these were recorded.

In the last five columns are recorded the number of days when ovipositions occurred. Table 1 shows only one such oviposition, but table 2 shows a much greater record. When the number of eggs laid were large and normal, the day on which it took place is merely given. When the eggs were few in number, this fact is also recorded; when subnormal to a degree an actual count of the eggs was made. By a normal number of eggs is meant batches varying between 50 and 100 or more eggs, while a few constitutes about 25.

Hutchison ('16) found that the house-fly females in captivity never lay more than two lots of eggs. The present writer repeated this observation in 1921 and thinks it in the main correct although Hewitt reported four batches per female in his experiments.

From the oviposition columns in the experiments on tables 1 and 2, it is impossible to attribute the number of depositions and the number of eggs to any particular female for the reason that isolated pairs were not used. The pairs were not isolated for two reasons. First, the writer confirmed the results of Hutchison ('16), who found that in experiments in which only one male and one female were used, the females often refused to lay; whereas the reverse happened in experiments containing a number of pairs. Hutchison found that only 24 per cent of isolated pairs deposited eggs in contradistinction to 63 per cent depositions

by a larger number of pairs. Hutchison calls this phenomenon a 'psychological factor' and calls attention to the observations of Bishopp, Dove and Parman ('15), who found that adult female house flies are in the habit of associating in large numbers at one particular spot for the purpose of oviposition. Hutchison further states, "the isolation of a pair of flies is an abnormal condition which has its inhibiting effect." Secondly, concentration of numbers of flies had other advantages and it really did not matter whether pairs were isolated or combined, so long as the same number of pairs was used, for the writer was interested in the effect of various types of food on the total number of ovipositions and the total number of eggs laid.

In the interpretation of the house-fly experiments, tables 1 and 2, the following facts are clearly discernible. When no food is tendered adult house flies in the summer, they die in from one to two days. When an almost pure protein diet is offered in the form of veal muscle bouillon, horse-blood serum or white of egg, the longevity is slightly increased although this type of food is clearly lacking in some essential requirement. The same thing may be said of bouillon and insoluble starch although in comparison with other starch foods, it is in experiment XIII not so much the lack of something as it is the nature of the food offered. The adult flies are unable to handle raw starch grains. This handling may be a mechanical difficulty for Graham-Smith and Hewitt have shown that the proboscis of the house fly is adapted only to the absorption of liquid or liquefied food. For further information on this point, the reader is referred to the extended and careful anatomical and experimental researches by the above mentioned authors. On the other hand, the question here, the handling of raw starch grains, may be a digestive matter with the fly.

When lump sugar (sucrose) is given alone or in conjunction with distilled water, a change is evident as may be seen by consulting columns '1 dead' and '6 dead'. In general the length of life of both the shortest and longest-lived individuals is increased appreciably. Also for the first time oviposition occurred, as is shown by experiment XVII in which six eggs were deposited on the thirteenth day.

Sucrose alone, then, in comparison with no food, a protein, or a protein plus an unbroken starch grain diet is an important factor in the longevity of house-flies. The influence of sucrose alone on oviposition is not so striking, but later experiments show that it is highly important.

The data on lump sucrose plus bouillon show that the longevity of the shortest-lived and longest-lived individuals is still further extended. Indeed this type of food, as well as one or two others to follow, reached all experimental records for the duration of life of summer flies. It is a well known fact, observed by Hewitt, Jepson and Griffith, that summer flies in captivity are shorter lived than winter generations. Jepson ('09) found that by rearing flies in February some individuals could be kept alive for eleven and a half weeks. In the summer, however, he was unable to keep flies alive in captivity for more than three weeks. Hewitt ('14), under the best food conditions, was never able to exceed seven weeks for his oldest captive house-flies. Hutchison found that the longevity of his summer and fall flies varied from 1 to 54 days with an arithmetical mean of  $19\pm$  days.

In the present writer's experiments on the most suitable foods for flies (experiments XIII to XXXV, inclusive), the longevity of the flies varied from 2 to 57 days, with an arithmetical mean of  $20\pm$  days. The longest-lived individuals had an arithmetical mean of  $34\pm$  days.

In so far as oviposition was concerned, a great many eggs were obtained with lump sucrose and bouillon. Since in experiment XVIII only males were used, no eggs were expected. It is interesting to note that in experiments XIX to XXII, inclusive, where double the ordinary number of females was used the number of ovipositions was far less than in experiments XXIII to XXVI, inclusive, in which equal numbers of both sexes are represented. Three of the last ones dead in the pure female lots were dissected and found full of eggs, so the inability to lay more in comparison with the females in the mixed sex lots seems to point to an egg-laying stimulus derived from the male sex. As will be shown later, this phenomenon was also observed in *Stomoxys*. It might be well to mention in this connection that many egg-hatching

tests were made with infertile eggs, i.e., eggs from pure female lots and with fertile eggs from mixed sex lots. The eggs from the pure sex lots, as expected, did not develop, so natural parthenogenesis may be safely excluded.

In general, the balance of the foods used (experiments XXVII to XXXV) compares favorably with lump sucrose and bouillon with perhaps the weight on the side of the latter combination. It is further interesting to note that whereas insoluble starch in suspension cannot be used by flies, soluble starch or hydrated starch in which the grains are broken by heat can be eaten and digested accompanied by an increase in longevity and by the production of eggs.

In 1916, Hutchison, in some work on the preoviposition period of the house-fly, found a variation from  $2\frac{1}{2}$  to 23 days. In the present writer's experiments in which ovipositions occurred in mixed sex lots, the preoviposition period varied from 11 to 24 days with an arithmetical mean of 15 days. This result seems to agree more closely with that of Hewitt ('07) who found the preoviposition period to be 14 days. Griffith ('08) found that 10 days were consumed by the preoviposition period while Bogdanow ('03) claims that 6 days is sufficient. Differences in food, temperature and humidity may have played a part in the production of the minor variations in the length of the preoviposition periods obtained by the writer and Hewitt on the one hand and Griffith and Bogdanow on the other. However, the writer questions Hutchison's minimum preoviposition period of  $2\frac{1}{2}$  days. Adult female flies on emerging must have ample suitable food and time for the full development of their ovaries and this, it seems, could rarely be accomplished in  $2\frac{1}{2}$  days. Dissections of females at intervals of 1 to 14 or 15 days performed by the author convinced him that Hutchison's result is very exceptional.<sup>2</sup> The length of the preoviposition period is undoubtedly influenced by temperature and humidity as Hutchison found, but it is also influenced by

<sup>2</sup> Moreover sexual activity on the part of the males usually occurred only after a number of days. Males and females in coitu were not observed in the experiments prior to the fifth day, although it may have taken place before unobserved.

food and probably by a stimulus derived from the male sex. The question of food and stimulus from the male sex are certainly noticeable in the duration and degree of oviposition.

By consulting the lowest and highest longevity columns of all experiments performed with mixed sexes, wherever sex mortality records were kept, it is apparent that female house-flies live longer than the males. This is not a striking point, but conforms with what we know of the longevity of male and female insects generally.

#### SUMMARY OF *M. DOMESTICA* EXPERIMENTS

1. In the summer house-flies reared in captivity without food die in from one to two days.
2. Reared house-flies live only a short while (one to eight days) on a diet of proteins, or products of protein hydrolysis and no eggs are laid.
3. Reared house-flies live only a short while (two to three days) on a raw starch diet and no eggs are laid.
4. On a diet of sucrose the longevity of the flies is increased, but no eggs are laid.
5. On a diet of sucrose and distilled water the longevity is approximately the same as in (4) and a few eggs are deposited.
6. On a diet of sucrose and bouillon, sucrose and blood serum, glucose and bouillon, glucose and blood serum, the longevity and degree of egg deposition reach their maximum.
7. On a diet of soluble starch and bouillon or hydrated starch and bouillon the longevity of house-flies is high and eggs are deposited.
8. On the most suitable foods the longevity of *M. domestica* varies from 2 to 57 days, with an arithmetical mean of 20+ days. The longest-lived individuals had an arithmetical mean of 34+ days.
9. The preoviposition period of *M. domestica* varies from 11 to 24 days with an arithmetical mean of 15 days.
10. Female house-flies when isolated from males lay fewer eggs than females that have associated with males.

11. As was expected, eggs laid by females in pure female sex lots did not hatch when placed in breeding jars on suitable media.
12. Eggs laid by females in mixed sex lots hatched when placed in breeding jars on suitable media.
13. In general female house-flies live longer than males.

#### CONCLUSIONS

From the experiments given it is justifiable to conclude that sugar or some form of starch that can be eaten and assimilated is an important factor in the longevity of adult house-flies. Also, sugar or assimilable starch, together with a solution of proteins or products of protein hydrolysis, like bouillon or blood serum, are necessary oviposition factors.

Types of food while important for the production of eggs are, however, not alone responsible for the degree of oviposition, i.e., for the number of depositions and total number of eggs. As has been shown, the male sex furnishes an important stimulus, the nature of which is unknown.

#### EXPERIMENTS WITH STOMOXYS CALCITRANS

By consulting table 3 (page 397) it can be seen that *Stomoxys* readily engorge on warm (35 to 37°C.) defibrinated whole cow or horse blood and that the longevity of the longest-lived individuals is quite high. The longevity varies from 3 to 46 days with an arithmetical mean of 20+ days. The longest-lived individuals had an arithmetical mean of 35+ days. This is approximately the same as the mean obtained for the house flies.

In the mixed sex lots, experiments I to VII, inclusive, one also notices that a large number of ovipositions occurred with the deposition of a large number of eggs. Experiments VIII to XI, inclusive, represent pure female sex lots and the same phenomenon occurred as was observed in the house-fly. In experiments VIII and IX no eggs were laid, and in experiments X and XI, only a very few were deposited. A number of the longest-lived females that finally died in these experiments were dissected and the ovaries were found full of ova, so again as in the house-flies the food given could not have been held responsible for the failure to

TABLE 3  
*Food experiments with Stomoxys calcitrans*

EXPERIMENT	FOOD	NUMBER DEAD AFTER . . . DAYS					OVIPositionS AFTER . . . DAYS						
		1 dead	2 dead	3 dead	4 dead	5 dead	First oviposition	Second oviposition	Third oviposition	Fourth oviposition	Fifth oviposition	Sixth oviposition	Seventh oviposition
I 3♂ 2♀	Defibrinated cow blood	8	11	16	16	23	9	10	11	14	18	21	
II 3♂ 2♀	Defibrinated cow blood	18	19	19	28	31	(few)	10	11	16	18	27	
III 3♂ 2♀	Defibrinated horse blood	7	13	15	17	42	10	11	13				
IV 3♂ 2♀	Defibrinated horse blood	20	26	29	41	46	9	10	11	12	18	27	
V 2♂ 3♀	Defibrinated horse blood	9♀	19♀	21♂	24♂	46♀	12	15	17	21	24	32	
VI 2♂ 3♀	Defibrinated horse blood	4♀	5♀	6♂	37♀	44♂	13	14	16	20	22	27 (few)	29
VII 2♂ 3♀	Defibrinated horse blood	18♂	18♀	23♀	34♀	39♂	11	13	15	16	17	21	23
VIII 5♀	Defibrinated horse blood	4	4	5	28	40	No ovipositions. Full of eggs	No ovipositions. Last two females that died					
IX 5♀	Defibrinated horse blood	10	12	17	20	23	No ovipositions. Last female that died full of eggs	No ovipositions. Last female that died full of eggs					
X 5♀	Defibrinated horse blood	6	22	22	26	38	20 (5 eggs)	26 (few)	No further ovipositions				
XI 5♀	Defibrinated horse blood	4	12	12	18	23	23 (2 eggs)	No further ovipositions. Last female that died full of eggs					

oviposit. Moreover, as was shown, oviposition occurred on the same food in mixed sex lots. The male again, as in the case of house-flies, exerts a stimulus on the females which influences them to lay.

The fertility of the eggs obtained in mixed and pure sex lots was tested by placing them on suitable media. Eggs from mixed sex lots hatched and second generations of *Stomoxys* were reared. The few eggs from the pure female experiments failed to hatch.

On defibrinated whole blood the preoviposition period of *Stomoxys* varied from 9 to 13 days with an artificial mean of 10+ days. This is slightly less than the mean for the house-fly preoviposition period, but the difference is probably due to the fact that the number of experiments performed with *Stomoxys* was less than the number of house-fly experiments from which it was possible to base the calculations.

On table 4 (XII to XXII, inclusive) are found experiments performed with two blood fractions, namely, one set with serum and another with the cellular elements, i.e., the red and white cells. It might be well to state that the flies completely engorged on either blood fraction.

In experiments XII to XVI, inclusive, the flies were fed with clear serum free from cellular elements. It will be seen by consulting the '1 dead' and '5 dead' columns that the longevity is less than is the case when flies are fed defibrinated whole blood. Furthermore, no eggs were laid.

In experiments XVII to XX, inclusive, the *Stomoxys* were fed simply with the cellular horse blood elements. This food was prepared by pipetting off the serum from centrifuged defibrinated blood and then washing and centrifuging three times with Ringer's solution. After the last centrifuging the upper Ringer's was again pipetted off and the cells in suspension in the remaining liquid used. By consulting the table, it is seen that in general the longevity of the flies was less than was the case when they were fed serum. Also no ovipositions occurred.

In experiments XXI and XXII, the two separately treated blood fractions were again combined and a return to normal was observed. Longevity was again increased and many eggs were laid.

TABLE 5  
Food experiments with *Stomoxys calcitrans*

EXPERIMENT	FOOD	NUMBER DEAD AFTER DAYS					OVIPOSITIONS AFTER DAYS				
		1 dead	2 dead	3 dead	4 dead	5 dead	First ovi-position	Second ovi-position	Third ovi-position	Fourth ovi-position	Fifth ovi-position
XII 2♂ 3♀	Horse blood serum	4♂	6♀	8♂	21♀	21♀					
XIII 2♂ 3♀	Horse blood serum	5♂	12♀	12♀	15♂	18♀					
XIV 2♂ 3♀	Horse blood serum	2♂	2♀	3♀	8♂	21♀					
XV 3♂ 2♀	Horse blood serum	4	11	12	12	17					
XVI 3♂ 2♀	Horse blood serum	5	5	5	7	12					
XVII 2♂ 3♀	Horse blood cellular elements	4♀	5♂	5♀	5♀	6♂					
XVIII 2♂ 3♀	Horse blood cellular elements	3♂	3♂	3♀	4♀	9♀					
XIX 3♂ 2♀	Horse blood cellular elements	2♀	4♀	7♂	9♂	9♂					
XX 3♂ 2♀	Horse blood cellular elements	3♂	3♀	6♂	8♀	17♂					
XXI 2♂ 3♀	Horse blood serum + washed cellular blood elements	6♂	17♂	17♀	19♀	25♀	13	15	16	17 (few)	19
XXII 2♂ 3♀	Horse blood serum + washed cellular blood elements	6♂	9♀	12♀	12♂	29♀	12	14	15	17	
XXIII 3♂ 2♀	Boillon	2	2	3	3	3					
XXIV 2♂ 3♀	Boillon + glucose	3	5	5	7	7					

Experiments XXIII and XXIV were simply performed to see whether *Stomoxys* would engorge on any other liquid food besides blood. As was expected they engorged, but the longevity was low and consequently no opportunity was offered for the development of the ovaries.

Lastly, an experiment not tabulated was performed. Two bottles containing each two male and three female *Stomoxys* were daily inverted on the shoulder of a cow. The flies were permitted to engorge after which they were again removed to the laboratory until the next day. Flies so treated and fed (fresh, undefibrinated blood) lived no longer nor produced more eggs than flies fed on warm, defibrinated blood which had been stored for a week or more.

#### SUMMARY OF *STOMOXYS CALCITRANS* EXPERIMENTS

1. Reared *Stomoxys* engorge readily on defibrinated, whole horse or cow blood when the latter is warmed to a temperature of 35 to 37°C.

2. The longevity of reared *Stomoxys* fed on defibrinated blood varies from 3 to 46 days, with an arithmetical mean of 20+ days. The longest-lived individuals had an arithmetical mean of 35+ days.

3. When fed defibrinated blood reared *Stomoxys* in mixed sex lots oviposit from three to seven times and lay many eggs.

4. Reared *Stomoxys* permitted to engorge daily on a cow (undefibrinated blood) live as long and lay as many eggs as flies fed defibrinated blood.

5. Female *Stomoxys* when isolated from males lay fewer eggs than females that have associated with males.

6. Eggs obtained from mixed sex lots hatch, develop and produce another generation of flies.

7. Eggs obtained from pure female sex lots do not hatch.

8. The preoviposition period of *Stomoxys* varies from 9 to 13 days with an arithmetical mean of 10+ days.

9. *Stomoxys* will engorge on either the serum or the cellular fraction of the blood.

10. On a diet of serum alone, the longevity is not as high as on a diet of defibrinated blood or when flies under experimental conditions are permitted to feed on cows. No eggs are laid.

11. On a diet of the cellular blood elements the longevity is very low. No eggs are laid.

12. When the two blood fractions are artificially combined again longevity and oviposition become normal.

#### CONCLUSIONS

Defibrinated cow or horse blood is an appropriate food for adult *Stomoxys*. On this food one can obtain many fertile eggs which will develop and produce another generation of flies.

In so far as longevity is concerned, the serum is probably the most important factor, but both the cellular elements and the serum are essential towards the production of eggs. The food, however, is not alone responsible for the degree of oviposition, i.e., for the number of depositions and total number of eggs. As has been shown with the house-fly, and can be more strikingly demonstrated in this form, *Stomoxys* males exert an important stimulus on the females, the nature of which is unknown.

#### EXPERIMENTS WITH *LYPEROSIA IRRITANS*

The work with the horn fly offered certain difficulties not foreseen at the start. Since *Stomoxys* was bred so easily, the writer considered that work with another bloodsucker would be rather more of a duplication or verification of results than anything else. This method of reasoning soon proved to be superficial, as is the case with so much biological theorizing from analogy. *Lyperosia* is a much more highly specialized parasite than *Stomoxys*. It is really a winged, obligatory, ectoparasite confining its attention exclusively to cattle on which it remains day and night, like a louse, with the exception that at intervals females with mature eggs dart down to freshly deposited cow dung for the purpose of oviposition. Having accomplished this function, they return to the cow where they either feed again or rest. *Stomoxys* is also an ectoparasite, but its parasitism has a wider range of hosts. It attacks horses, mules, cattle, sheep and hogs; frequently

poultry, and even man at times. *Stomoxys*, moreover, does not remain on its host continuously, but after engorgement usually flies off to some more or less distant place to oviposit or to some undisturbed place for the digestion of its meal. This species also breeds in a greater variety of media such as horse manure, fermenting straws of various sorts and according to Bishopp ('13) it has been found to breed in cow-lot manure when mixed with waste feed and in ensilage.

The writer does not wish to convey the impression that he offers higher specialization as an explanation for certain difficulties encountered in his experiments with *Lyperosia* but merely wishes to imply that specialization creates difficulties; it does not explain them.

The matter of rearing *Lyperosia irritans* beyond the first generation is still a problem, but some progress has been made and therefore the results had best be presented. Judging from the literature, no one seems to have attempted to rear this species.

On tables 5 and 6 are found the results obtained with the horn fly. These two tables do not appear quite as neat as the others for the reason that the writer was often compelled to 'make up' sex lots in different proportions. This was due to the fact that on the predicted day of emergence a special kind of food had been prepared and the flies had to be used as soon as they appeared. Now, it often happened that on one day very few adults appeared, and on the other very many issued. Still, the flies had to be fed in spite of large or small numbers or a disproportion of sexes, and so, in order to use the new flies and give them the benefit of the fresh food, much juggling had to be resorted to and the result is the appearance of the two tables. However, the facts are there disclosed and are in no way influenced by the number of flies used or the proportion of the sexes.

In 1921, the writer noticed that when horn flies were taken from cattle and placed in breeding jars, they laid many eggs. As a matter of fact, gravid females very often oviposited in the collecting bottles before an opportunity was given them to lay on a proper medium. Eggs laid in the breeding jars on cow dung hatched, the larvae developed, and the first generation of reared

*Food experiments with Liprostia virilans*

EXPERIMENT	FOOD	NUMBER DEAD AFTER DAYS												OVIPOSITIONS AFTER DAYS			
		1	2	3	4	5	6	7	8	9	10	11	12	First ovi-position	Second ovi-position	Third ovi-position	Fourth ovi-position
I 6 wild ♂ 6 wild ♀	Deformed raw blood	1 dead	2 dead	3 dead	4 dead	5 dead	6 dead	7 dead	8 dead	9 dead	10 dead	11 dead	12 dead	2 (many)	3 (4 eggs)		
II 6 wild ♂ 6 wild ♀	Deformed raw blood	4	4	7	7	7	10	11	14	15	21	22	25	2 (many)	3 (many)	4 (10 eggs)	5
III 6 wild ♂ 6 reared ♀	Deformed raw blood	3	9	9	10	11	13	15	18	18	19	19	21	13 (1 pale egg)			
IV 6 wild ♂ 6 reared ♀	Deformed raw blood	3	4	4	8	8	9	9	9	14	14	14	30	17 (15 pale eggs)	23 (3 pale eggs)		
V 2 ♂ 3 ♀	Deformed raw blood	8	9	9	11	21											
VI 2 ♂ 3 ♀	Deformed raw blood	10	10	10	12	13											
VII 5 ♂ 5 ♀	Deformed raw blood	8	8	8	9	10	10	11	12	12	21			Killed ♂, not fertile	Both testes normal.	Spermatocytes	
VIII 5 ♂ 5 ♀	Deformed raw blood	6	8	9	10	12	15	15	17	17	123						
IX 2 ♂ 3 ♀	Deformed raw blood	6	7	14	17	20											
X 2 ♂ 3 ♀	Deformed raw blood	7	8	16	21	27								Both ovaries normal.	Full of early ova		
XI 2 ♂ 3 ♀	Deformed raw blood	9	11	12	16	21								Both ovaries normal.	Few slightly active spermatocytes		
XII 2 ♂ 3 ♀	Deformed raw blood	9	9	9	17	17								Both ovaries normal.	Full of early ova		
XIII 2 ♂ 3 ♀	Deformed raw blood	7	10	16	21	25								Killed ♂.	Both testes normal.	Few slightly active spermatocytes	
XIV 2 ♂ 3 ♀	Deformed raw blood	5	6	19	20	23											

\* ♀♀ Killed and dissected. Both ovaries normal. Well developed ova.

TABLE 8  
Food experiments with *Luperosia irritans*

EXPERIMENT	FOOD	NUMBER DEAD AFTER DAYS											
		1	2	3	4	5	6	7	8	9	10	11	12
		dead	dead	dead	dead	dead	dead	dead	dead	dead	dead	dead	dead
NV 6♂ 6♀	Citrated cow blood	2	2	2	2	3	6	6	12	16	17	17	17
NVI 6♂ 6♀	Citrated cow blood	2	2	3	11	11	12	13	18	18	21	21	21
NVII 3♂ 4♀	Citrated horse blood	4	6	9	11	11	12	12					
NVIII 3♂ 4♀	Citrated cow blood + glucose	12	12	14	14	17	19♂	19♀					
IX 3♂ 4♀	Citrated cow blood + glucose	7	14	14	17	20♂	20♂	20♀					
XX 3♂ 4♀	Defibrinated cow blood + glucose	7	14	14	14	16	19	20					
XXI 3♂ 4♀	Defibrinated cow blood + glucose	7	14	14	15	15	15	21♀					
XXII 3♂ 4♀	Defibrinated horse blood + glucose	2	11	12	13	15	21	21♀					
XXIII 3♂ 4♀	Defibrinated horse blood + glucose	2	7	14	15	15	15	22♀					

XXIV 6♂ 6♀	Defibrinated cow blood + ear extract	2♂	3♀	7♂	9♀	14♂	14♀	17♀	19♀	19♂	19♀	20♀	21♂
XXV 6♂ 6♀	Defibrinated cow blood + ear extract	2♂	3♀	10♀	10♀	14♂	16♀	17♂	17♂	18♂	18♂	21♀	22♀
XXVI 6♂ 6♀	Defibrinated cow blood + liver extract	5♂	5♀	6♀	6♂	6♂	6♀	7♀	9♂	13♂	15♂	16♀	17♀
XXVII 6♂ 6♀	Defibrinated cow blood + liver extract	2♀	3♂	3♀	6♂	6♂	9♀	10♀	13♂	15♀	15♂	16♀	17♂
XXVIII 6♂ 6♀	Defibrinated cow blood + alfalfa extract	2♀	4♀	6♀	9♂	10♂	12♀	15♂	15♀	16♀	17♂	18♂	19♂
XXIX 6♂ 6♀	Defibrinated cow blood + alfalfa extract	2♀	4♀	7♂	8♀	8♀	9♂	9♂	10♂	12♂	13♂	13♀	24♀
XXX 6♂ 6♀	Defibrinated cow blood + Ringer's solution	2♀	7♂	8♀	9♂	9♀	10♂	10♂	12♂	17♂	19♀	21♀	22♀
XXXI 6♂ 6♀	Defibrinated cow blood + Ringer's solution	3♀	6♀	7♂	8♂	8♀	8♂	10♀	13♀	18♂	19♂	22♀	23♂

adults was obtained. From 'this point on,' however, the story began to differ from that of *Stomoxys* and the house-fly. First generation reared adults artificially fed consistently failed to lay eggs and, therefore, it has been impossible to rear a second generation.

In 1922, the writer planned a large series of experiments for the purpose of throwing light on this interesting situation. The results of these experiments did not clear up the matter, but a number of interesting things were, nevertheless, unfolded.

Experiments I and II (table 5) are typical representatives of what occurs when wild males and females are taken from cattle, placed in bottles and fed twice daily with defibrinated, warm cow blood. The flies although they engorge and remain alive in captivity for from two to twenty-five days, cease laying eggs in from three to five days.

To determine, if possible, which sex was at fault experiments III and IV were performed. In these, wild males were placed in bottles with first generation reared virgin females and fed defibrinated cow blood. Reared males were not mated with wild females because it is impossible to determine without sacrificing the females whether impregnation has occurred or not. In experiment III one pale egg was deposited on the thirteenth day, and in experiment IV fifteen pale eggs were deposited on the seventeenth day, and three on the twenty-third day. These nineteen eggs collapsed in a few days and failed to hatch on cow dung. The two experiments demonstrate nothing in regard to the males, but they show that the females are affected either by the food, treatment, or both. In the first place, the females laid very few eggs and in the second place the chorion was devoid of all pigment. The normal eggs of wild *Lyperosia* are a brown color. The two last females in experiment IV were killed and dissected on the thirtieth day. The ovaries appeared normal and contained early ova. Apparently most of the ova progressed up to a certain point and then development was arrested.

The remaining experiments on table 5 (experiments V to XIV, inclusive) represent mixed cultures of first generation reared males and females fed either defibrinated cow or horse blood.

No eggs were deposited in any of the test bottles. In experiment VII, the last fly alive happened to be a male. Both testes were removed, examined and found superficially normal. These organs were triturated in warm physiological salt solution and it was found that the spermatozoa were inactive. The testes of normal wild males were compared at the same time and in these the mature spermatozoa showed activity by way of undulations. Similar observations were made in experiments XI and XIII, with the exception that a few slightly active spermatozoa were seen. The activity, however, was not at all like the agitation found among the spermatozoa of the control wild flies examined at the same time. Judging by the age of the experimental males and from cytological evidences, it seemed that spermatogenesis had progressed normally and had had ample time. In experiments X and XII, the last females dead were examined. The ovaries seemed normal and were full of early ova.

The citrated whole blood foods given on table 6 contained 0.1 per cent of sodium citrate. Ammonium citrate was first used on another set of flies, but they would not eat blood so treated. However, the flies regularly engorged on blood containing the sodium citrate. When glucose was added to the citrated whole blood or defibrinated blood, this was done by adding 1 cc. of a strong glucose solution to 10 cc. of blood. Glucose was added in experiments XVIII to XXIII, inclusive, for the reason that this sugar is the one found in normal blood and the possibility existed that it might have been gradually 'broken down' after the blood had been removed from the cow or horse. An attempt was merely made to restore to the blood one of the constituents that might have undergone a change. As can be seen from the tables, no ovipositions occurred as a result of the use of this food.

The possibility also existed that the flies under normal conditions obtain certain tissue extracts that affect reproduction. In puncturing the skin of a cow, the proboscis of the flies before reaching the capillaries undoubtedly often penetrates through a number of tissues, such as skin, connective tissue, and possibly muscle. Extracts derived from these tissues might have a stimulating effect upon the sex glands and reproductive organs gener-

ally. Therefore, extracts from cow-ears and cow-livers were prepared and added to defibrinated cow blood. The external ears were chosen because here in one organ a number of tissues are represented such as skin, fascia, and muscle. Five drops of an aqueous extract to 10 cc. of defibrinated blood were daily given, but with these foods, as can be seen from experiments XXIV to XXVII, no eggs were obtained.

In experiments XXVIII and XXIX alfalfa extract, in the same proportion as the tissue extracts, was administered daily with the blood, but no eggs were laid. Alfalfa is known to be rich in vitamins and was used for this reason.

It was also thought that possibly the addition of various salts, particularly calcium, might have a stimulating effect so the last two experiments were performed by adding Ringer's solution to defibrinated cow blood. The result demonstrated nothing of importance.

The reared flies when artificially fed with the various types of foods lived from 1 to 24 days. The longest-lived individuals had an arithmetical mean of 20 days.

Judging from the few abnormal pale eggs, laid in experiments III and IV, on table 5, the preoviposition period of the horn-fly lies somewhere near the thirteenth or seventeenth day after emergence.

#### SUMMARY OF *LYPEROSIA IRRITANS* EXPERIMENTS

1. *Lyperosia irritans* gravid females when removed from cattle and placed in breeding jars lay many eggs.
2. *Lyperosia*, up to the present, cannot be reared artificially beyond the first generation of adults.
3. First generation reared adult females in captivity lay no eggs.
4. Wild males and females taken from cattle and fed twice daily with defibrinated, warm cow blood remained alive in captivity for from 2 to 25 days, but cease laying eggs in from 3 to 5 days.
5. Experimental evidence is advanced to show that the reproductive organs of the reared and artificially treated females are affected.

6. Observational evidence is advanced to show that the reproductive organs of the reared and artificially treated males are affected.

7. Reared *Lyperosia*, artificially fed, live from 1 to 24 days. The longest-lived individuals had an arithmetical mean of 20 days.

8. From two experiments it was possible to show that the pre-oviposition period of *Lyperosia* probably does not lie far from the 13th or 17th day after emergence.

#### CONCLUSIONS

Although the first generation reared *Lyperosia irritans* can be kept alive under artificial conditions long enough for the development of the ovaries and testes, they lay no eggs. For this reason it has been impossible to rear a second generation of flies. Whether the food, the treatment, or both are responsible, it is difficult to conclude.

The reproductive organs of both sexes are affected, and it might be well to mention also an effect upon the nervous system, for sexual excitation and copulation have never been observed among any of the author's reared *Lyperosia* adults.

#### GENERAL DISCUSSION

It was seen from all of the foregoing work that flies are dependent upon certain types of food which are closely correlated with longevity and reproduction. It must not be assumed, however, that the foods tendered were the only things that found their way into the intestinal lumen. The part played by various intestinal bacteria was not touched upon in this study. Flies carry over bacteria from the larval to the adult stage, as shown by Bacot, Graham-Smith, and others. This is especially true of house flies which, as adults, also ingest microorganisms. These bacteria many of which multiply within the intestines may be the source of most of the vitamin requirements of adult flies. Of course, the flies may obtain enough vitamins in their larval food to carry them along as adults, but this does not necessarily follow. Then again, some of the bacteria found in adults may play more or less of an accessory digestive part (symbiosis) or may be more or less

pathogenic at times. Any of these possibilities may have an important bearing on longevity and reproduction.

In the experiments outlined, no account was taken of the variations in temperature and humidity occurring at different times during the season. Such variations are known to have an effect on longevity and reproduction in other insects, as shown by Pierce's work on aphids. They may have had a profound effect on *Lyperosia*.

Pearl and Parker ('22) intensively studied the duration of life of the fruit-fly, *Drosophila melanogaster*. These authors dealt with the effects of successive etherizations, density of population, and ventilation on longevity. Such factors could not have played a rôle in the work described by the present writer, because with the exception of differences in the food all experiments were performed in the same way. The flies were only etherized once, only a few individuals were placed in each bottle, and the ventilation was good.

The writer was primarily interested in the effect of food, and this interest arose out of practical considerations. He wished to raise large numbers of healthy flies and keep them alive for disease studies. Reasonable precautions were taken to avoid undue bacterial contaminations and great fluctuations in temperature and humidity, but nothing more. Whether other factors have an important bearing on longevity and reproduction or not, need not cause much disturbance to the results obtained in the present work. The food issue is defined sharply enough, especially, in the case of the house-fly and *Stomoxys* so that other variables when considered in the future will have a tendency to accentuate the results here presented rather than detract from them.

For summaries and conclusions on the work accomplished with the three species of flies used, the reader is referred to the end of the sections dealing with the house-fly, the biting stable-fly, and the horn-fly respectively.

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## ANESTHETICS AND CO<sub>2</sub> OUTPUT

### II. DECREASED CARBON DIOXIDE OUTPUT AND RECOVERY FROM ETHER IN CERTAIN ORTHOPTERA

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#### FIVE FIGURES

That anesthetics have marked effects upon the carbon dioxide output and the oxygen intake of organisms has been repeatedly pointed out by various investigators, Osterhout (1), Haas (2), Gustafson (3), Brooks (4), Thomas (5), Irwin (6), Smith (7), Inman (8), Bodine (9), and others. In order to study the action of these chemicals upon organisms more fully, particular attention has recently been given to the initial and recovery stages of anesthesia (7, 10). It has been shown for example, that ether, in the initial stages of its action, produces in the case of plants and some animals an initial lowering of the rate of carbon dioxide output followed by a rapid increase and this in turn is succeeded by a fall. In grasshoppers, however, no initial decrease in the rate of carbon dioxide output in ether was detected (9). Recovery from anesthetics in the case of plants has been shown to be of two types, partial and complete (8, 10). In the former a persistent lowering of the rate of carbon dioxide output takes place while in the latter the organism returns to its normal rate of carbon dioxide output when removed from the action of the anesthetic. As a result of these investigations various important questions as to the action of anesthetics have arisen. For example, just how far can an anesthetic have a stimulating effect and just when does anesthesia begin and when do irreversible changes take place.

Since no results for animals like grasshoppers seem to be available, it was thought desirable to study in some detail the various

changes in the rate of carbon dioxide output undergone by different species of these animals during the stages of ether anesthesia as well as during recovery. The present paper embodies results obtained from such investigations.

The material used in these experiments was various species of grasshoppers most of which were reared entirely under the usual laboratory conditions. Nymphs or adults of the following species were used: *Chortophaga australior*, *Chortophaga viridifasciata*, *Melanoplus femur-rubrum*, *Melanoplus differentialis*, *Arphia xanthoptera*, *Encoptolophus sordidus* and *Dissosteira carolina*. These organisms have been found to be rather favorable material for respiration experiments because body movements can be practically eliminated by proper handling and manipulation of the animal. It has also been found that decapitated animals can be used as readily as normal ones thus eliminating to a great extent such factors as muscular tone as well as possible light stimulation, etc. Results from normal and decapitated animals, have been found, in the case of ether, to be qualitatively similar.

Carbon dioxide determinations were made by the indicator method described in a previous paper (9). Briefly, the method is based upon the comparison of the color change of phenol-sulfonephthalein in the experimental tubes with that in standard tubes containing known amounts of carbon dioxide. By using a series of standard tubes containing graded amounts of carbon dioxide, the rate of carbon dioxide production by the animal from minute to minute can be conveniently followed. Reagents are added to both experimental and control tubes. Pyrex or non-sol tubes, 25 cc. in capacity were used throughout experiments. The average room temperature was 23°C.

In expressing results, the rate of respiration in each case is expressed as per cent of the normal rate (which is always taken as 100 per cent).

The concentration of ether used varies in different experiments. It has been previously pointed out that rather small doses of ether are sufficient to keep the animals anesthetized for long intervals of time (9). Similar doses for different periods of time as well as

different doses for the same time periods were used and are indicated in the descriptions of experiments which follow. Animals in all experiments were exposed only to the vapors of the reagent.

Temperature during any experiment was kept practically constant, varying not more than 0.5°C.

Figure 1 shows graphically the changes in the rate of carbon dioxide output typical for an animal when exposed to a reversible dose of ether;—a marked initial increase followed by a decrease and this in turn succeeded by an almost constant rate of carbon

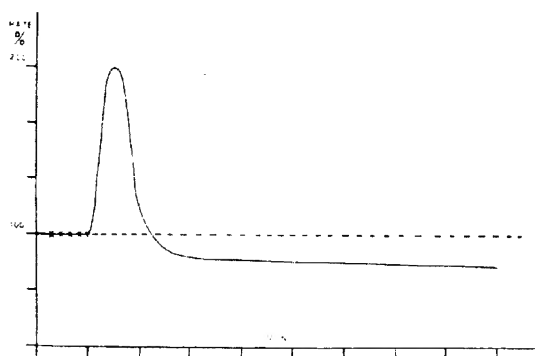


Fig. 1. Curves showing the effect of ether, a reversible dose (four drops to 0.16 cc.) on the CO<sub>2</sub> production of grasshoppers. The point marked O on the abscissa indicates the beginning of exposure to ether; previous to this the normal production of CO<sub>2</sub> was determined which is taken as 100 per cent. The ordinates denote the rate of production of CO<sub>2</sub> expressed as per cent of the normal, the abscissa, time in minutes. Curve represents the average obtained from thirty-five individuals. For further explanation see text.

dioxide output. The fact that a constant rate of carbon dioxide output maintains (in reversible doses) doubtless points to the conclusion that an equilibrium between the organism and the ether (at that concentration) has been established. No more ether is taken up by the animal once this equilibrium is established at a definite per cent of the reagent. If, however, the concentration of the ether is increased to that of an irreversible dose more ether is taken up as indicated by a marked constant drop in the rate of carbon dioxide output. Shafer (11), also pointed

out that in the case of a beetle, *Passulus cornutus*, no more ether was taken up by the animal at any per cent of the chemical after equilibrium was once established.

The following general method was observed in recording the rates of carbon dioxide output during the recovery of animals from ether. Animals were subjected to reversible and irreversible doses for varying lengths of time ranging from two minutes to one-half hour. They were then taken out of the ether and the rates of carbon dioxide output followed until complete recovery occurred. During this process of recovery, various important changes in the rate of carbon dioxide output, due to the rate at which different regions of the animal recovered from the anesthetic, were noted. Shafer (11), has shown that ether taken up by the tissues of an insect comes out at a slower rate than it enters. In order to rule out the effects of any ether which came out of the grasshoppers while in the experimental tube various experiments were tried. Among them the following may be noted. Etherized animals taken from the etherizing tube were put for various periods of time, corresponding to the lengths of time they were kept in any one tube during a respiration experiment, into empty tubes and the tubes corked. Normal animals were then subjected to this small amount of ether which had escaped and it was found that no appreciable effects on their rates of carbon dioxide output could be detected, thus showing that in any recovery experiments the amounts of ether released from the tissues of an animal while in the respiration tube were not sufficient to appreciably modify the rate of carbon dioxide output during recovery experiments.

Since the results obtained in these experiments are based upon large numbers of animals and are qualitatively alike, the data can be best given by showing typical cases.

Figure 2 shows graphically the recovery of animals after different lengths of exposure to ether. An examination of these curves shows that animals subjected to the reagent for an extremely short time, two or three minutes (and this period corresponds to the rapid rise in the rate of carbon dioxide output in the animals while in the ether (reversible dose) as indicated in figure 1), quickly return to the normal rate of carbon dioxide out-

put when taken out of the anesthetic. Respiratory movements in many such experiments did not cease and were not appreciably different in rate from the normal, thus showing that this increased rate of carbon dioxide output followed by a gradual return to normal could not be accounted for by changes in respiratory movements. After five minutes' exposure to the ether, however,

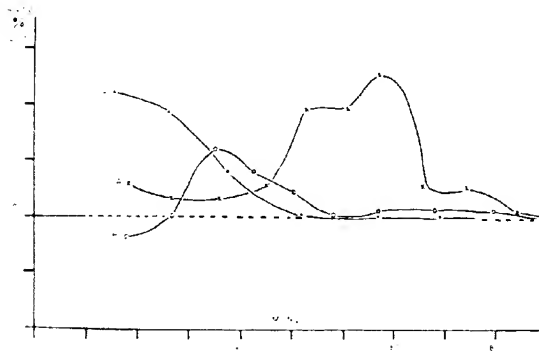


Fig. 2 Curves showing the rate of CO<sub>2</sub> output of animals after treatment with reversible doses of ether. The first point on each curve denotes the per cent to which the rate of CO<sub>2</sub> output had reached when the animals were transferred from the ether to air. The ordinates denote the rate of production of CO<sub>2</sub> expressed as per cent of the normal, the abscissae, time in minutes. Each curve represents a typical experiment.

Curve A shows recovery of animal from 0.16 cc. of ether for five minutes. Respiratory movements ceased. The first rise in curve due to return of respiratory movements,—the second due to reflex movements.

Curve B same as curve A, except for another species of animal.

Curve C shows recovery of animal from 0.16 cc. of ether for two minutes. Respiratory movements did not cease. For further explanations see text.

the animal also quickly revives but the recovery is somewhat complicated by other factors. Recovery toward the normal rate of carbon dioxide output occurs before any detectable movements of the animal take place. Then follows a slight rise in rate of carbon dioxide output due to the return of respiratory movements. A few minutes after respiratory movements begin a still greater increase in rate of carbon dioxide occurs due to violent tremors which come over the animal. Movements are apparently

not at all inhibited by the central nervous system or brain at this stage of recovery. A short interval after this other movements dependent upon the functioning of the central nervous system or brain occur and the rate of carbon dioxide output then quickly returns to the normal.

Figure 3 shows graphically the recovery from reversible and also the partial recovery from irreversible doses of ether. From this figure it is evident that in animals where partial recovery

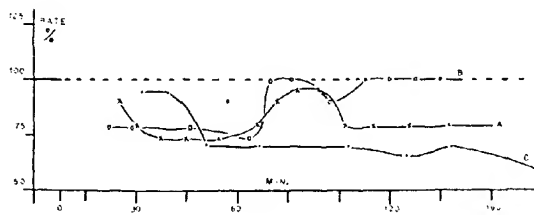


Fig. 3 Curves showing the rate of  $\text{CO}_2$  output of animals after treatment with reversible and irreversible doses of ether. The first point on each curve denotes the per cent to which the rate of  $\text{CO}_2$  output had reached when the animals were transferred from the ether to air. The ordinates denote the rate of production of  $\text{CO}_2$  expressed as per cent of the normal, the abscissae, time in minutes. Each curve represents a typical experiment.

Curve A shows irreversible effect of 0.16 cc. of ether for twenty minutes. A partial recovery of respiratory movements occurred as indicated by rise in rate of  $\text{CO}_2$  output.

Curve B shows recovery of animal from 0.16 cc. of ether for fifteen minutes. Note rise in rate of  $\text{CO}_2$  output due to return of respiratory and reflex movements.

Curve C shows complete irreversible effect of 0.16 cc. of ether for thirty minutes. For further explanations see text.

occurs, as in the case of partial return of respiratory movements, changes in the rate of carbon dioxide output similar to those for reversible doses at this stage also take place.

That the above described changes in the rate of carbon dioxide output are not dependent or due to body movements, etc., is clearly brought out in figure 4, showing a typical result for a decapitated animal recovering from a reversible dose of ether.

The animal when decapitated has a lowered rate of carbon dioxide output (due doubtless to loss of muscular tone, etc.) but when subjected to ether and allowed to recover changes in

the rate of carbon dioxide output occur much as for the normal animal, i.e., in the case of reversible doses the rate of carbon dioxide output gradually returns to the normal for a decapitated animal.

Figure 5 shows graphically the incomplete recovery of animals subjected to fumes of ethyl alcohol. An almost constant decreased rate of carbon dioxide with no tendency to a return to the normal as in the case of ether occurs. Grasshoppers are apparently quite susceptible to fumes of ethyl alcohol.

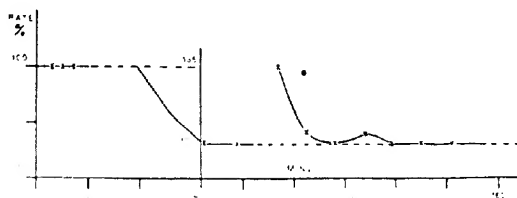


Fig. 4—Curves showing the rate of CO<sub>2</sub> output of a normal animal; the animal after decapitation and then the recovery of the decapitated animal from 0.16 cc. of ether for five minutes. The ordinates denote the rate of production of CO<sub>2</sub> expressed as per cent of the normal, the abscissae, time in minutes. Left hand side of middle ordinate shows normal rate of CO<sub>2</sub> output for intact animal and fall in rate due to decapitation. Right hand side of middle ordinate shows recovery of decapitated animal from ether. For further explanation see text.

That recovery from ether is not identical to that of other reagents and that various chemicals react differently can be better shown by a comparison with recovery from cyanide. In a subsequent paper on the effects of cyanide on grasshoppers some points relative to this topic will be presented.

The question of the stimulating and narcotic effect of ether is of much interest. It would seem, from a consideration of the recovery of grasshoppers from the chemical, that stimulation doubtless takes place primarily during the time the marked increase in the rate of carbon dioxide output occurs and narcosis when the decline to a constant rate follows. An animal subjected to ether for two to three minutes (a reversible dose) shows no appreciable change in respiratory movements, appears quite normal and is not anesthetized. A decided increase in rate of

carbon dioxide results and a rapid and marked decrease to normal occurs when the animal is returned to air. It is quite conceivable that during this stimulating period the anesthetic exerts some effect on the cell membrane, with consequent permeability changes, since recovery is then most rapid. Narcosis on the other hand, conceivably might occur when the anesthetic actually enters the cell or produces marked permeability changes, for it is at this time in the progress of the etherization that recovery is relatively longest. That such changes might occur

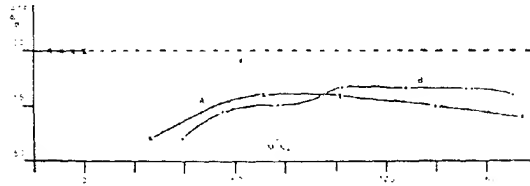


Fig. 5 Curves showing the rate of  $\text{CO}_2$  output of animals after treatment with fumes of ethyl alcohol. The first point on each curve denotes the per cent to which the rate of  $\text{CO}_2$  output had reached when the animals were transferred from the alcohol fumes to air. The ordinates denote the rate of production of  $\text{CO}_2$  expressed as per cent of the normal, the abscissae, time in minutes. Each curve represents a typical experiment.

Curve A shows irreversible effect of exposure to alcohol fumes for seven minutes. Curve B shows irreversible effect of exposure for thirty minutes. For further explanation see text.

are undoubtedly suggested by the results of Shafer (11) who found that at certain percentages of ether only a definite amount of the reagent is taken up while the animal is alive. Future experiments, however, must definitely decide just to what extent and when stimulation occurs and when narcosis begins and also what determines an irreversible change in the organism.

Inasmuch as all degrees of recovery seem possible in grasshoppers (depending upon length of exposure and dose) the results above obtained are strikingly similar to those for plants obtained by Osterhout and his students (12).

## SUMMARY

1. Marked changes in the rate of carbon dioxide output in grasshoppers are produced by ether.

2. Changes in the rate of carbon dioxide output of animals removed from reversible and irreversible doses of the reagent are pointed out.

3. Recovery from ether may be partial or complete.

4. The order in which various parts of an animal recover from ether is: first the respiratory movements, then movements usually controlled or inhibited by the brain and finally the brain itself.

5. Further investigations are necessary to show when stimulation begins and ends and when narcosis and irreversible changes occur in animals subjected to ether.

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THE INHERITANCE OF CINNABAR EYE-COLOR IN  
*DROSOPHILA MELANOGASTER*, INCLUDING  
DATA ON THE LOCUS OF JAUNTY<sup>1</sup>

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During the summer of 1920 I became interested in the possibility of securing from nature variant forms of *Drosophila melanogaster* similar to those which had been discovered as mutants in laboratory cultures. In order to obtain data on this point I trapped a number of *melanogaster* individuals at various places in Berkeley and reared their progeny in the laboratory to see whether or not segregation occurred in them. In only one case were any new forms secured by this method, but the number of individuals tested was not large. Later, so many new forms appeared in experimental cultures that the task of studying them adequately became of more importance than the original problem.

In the one successful case a single wild-type female was trapped in Strawberry Canyon on the grounds of the University of California on August 16, 1920. She was immediately transferred to a culture bottle in the laboratory, without addition of a male fly, since she had evidently mated previous to capture. Her progeny was given only a casual examination, but it seemed to consist entirely of wild-type flies. A mass culture of these flies was established. The second generation of progeny exhibited segregation for two new primary eye-colors: one a bright eye-color similar in appearance to vermilion, another a dark eye-color, somewhat like safranin; and some flies had an eye-color apparently due to combination of these two colors. Some of

<sup>1</sup> The writer is indebted to Dr. C. B. Bridges for his kindness in reviewing this paper while it was in manuscript form and for offering valuable suggestions in the treatment of the data.

the females of this second generation were also found to have short, slender bristles associated with irregular markings on the abdomen. Although no counts were made of this mass culture, the three types of individuals appeared in such large numbers as to leave no doubt that they were the result of segregation rather than of original mutation.

Stocks were established of all three types, but the stocks naturally each continued to segregate for the other characters. Of the three characters obtained, the vermillion-like eye-color, named cinnabar at the suggestion of Dr. C. B. Bridges, was by far the most striking and promising one, and experiments were immediately initiated to determine its genetic relations. These experiments are reported in detail in the present article.

The dark eye-color was found by the customary method of group determination to be a simple recessive character dependent upon a third-group gene. In our experience, however, it does not differ strikingly enough from wild-type to be valuable for genetic work, because there are always a number of flies which are difficult to classify. Crossed with star dichaete it gave the four customary classes in  $F_1$  for a recessive character, and the back-cross of  $F_1$  star dichaete males to dark females gave star dichaete, star dark, dichaete, and dark in approximately equal numbers, the evidence for location of its gene in the third-group. Its locus within the third-group has not been determined.

The short-bristled, scaly-abdomen type when obtained in stock proved to be sex-limited. The females exhibit the character, but the males are indistinguishable from wild-type. It also proved to be sex-linked. From conversations with Dr. A. H. Sturtevant, it seemed possible that 'scaly,' as it was then called, was identical with 'bobbed,' a previously discovered sex-limited, sex-linked mutant. In order to test this possibility it was crossed with 'bobbed' from the Columbia stocks. The female progeny were all 'bobbed' which seemed to be sufficient proof of identity of the two forms. It seemed unimportant, therefore, to continue work with it.

## STOCK OF CINNABAR

The cinnabar stock as originally established was obviously contaminated both with 'bobbed' and with the 'dark' eye-color. When cinnabar was found to belong to the second group, however, it became an easy matter to free it from these forms, the groups of which were then also known. The procedure adopted for 'purifying' it was as follows. A star dichaete female from star dichaete stock was mated to a male from the cinnabar stock. An  $F_1$  star dichaete male from this mating was then out-crossed to a female from a wild stock. A dichaete male and female from the progeny of this cross were then mated for the production of 'purified' cinnabar. It is obvious that this system of matings had the effect of replacing the X- and III-chromosomes of the original cinnabar stock with chromosomes known to contain only wild-type genes, and there was also a possibility that the IV-chromosome had been replaced, although this was probably not important. The II-chromosome was retained in its original condition. This stock of cinnabar was then used as a basic stock for making up all cinnabar combinations. Cinnabar, thus established, did not subsequently exhibit the marked variability in eye-color characteristic of the original stock, doubtless due to the elimination of dark, and it never produced 'bobbed' segregants.

## DESCRIPTION OF CINNABAR

Cinnabar, symbol *en*, is so nearly identical with vermilion in appearance, as its name suggests, that no detailed description of it is necessary. It is readily separable from wild-type with the unaided vision. The color is particularly intense in young flies and becomes somewhat darker with age. The ocelli are colorless as in vermilion and scarlet, a characteristic which is sometimes of aid in classification, especially with very old flies. It adds a third member to the vermilion 'genus' of characters, of which scarlet, discovered by Mrs. Richards ('18) and by Lancefield ('18) is the second.

## THE LINKAGE-GROUP OF CINNABAR

The linkage-group of cinnabar was determined in the customary manner by using star and dichaete as markers of the second and third groups, respectively. From cinnabar ♀ by star dichaete ♂ an  $F_1$  progeny consisting of approximately equal numbers of star dichaete, star, dichaete, and wild-type flies was secured. Five single pair cultures of  $F_1$  star dichaete ♂ ♂ by cinnabar ♀ ♀ were established, and yielded progeny distributed approximately equally among four classes; star dichaete, star, dichaete cinnabar, and cinnabar, as shown in table 1. The distribution obviously is typical of a second-group mutant.

## THE LOCUS OF CINNABAR

As a first approximation to the locus of cinnabar, its recombination per cent with star was determined. For this purpose star females were isolated from the cultures listed in table 1 and mated to cinnabar males from stock. The data are recorded in table 2. The percentage of recombination (omitting aberrant no. 76) in these cultures was 40.3, which indicates a locus slightly to the right of black, since the standard star black recombination per cent is 37.9.

Since cinnabar is a character of the first rank in respect to viability and separability, it seemed important to determine its locus more accurately in relation to genes located in the middle region of the II-chromosome. At that time among the stocks which we had on hand the characters black and vestigial appeared to be most suitable for the purpose, since the locus was apparently somewhere between those of these two genes. Accordingly, the various combination stocks necessary for carrying out the four back-cross determinations of the three-point method were made up. Since no difficulties were encountered in securing them it seems unnecessary to describe the methods employed, except to state that the stocks so established contained only the mutant genes indicated in the tables, and they were not complicated by the presence of other genes, segregation for which might have obscured the results.

The data obtained from the various back-crosses are presented in tables 3, 4, 5, and 6. The tables are self-explanatory. Single pair cultures were used throughout. A summary of the data from these tables together with recombination per cents is given in table 7. Two striking features of these data are the low aver-

TABLE 1  
*P*<sub>1</sub>, *star* *dichaete* ♂ × *cinnabar* ♀; *B.C.*, *P*<sub>1</sub> *star* *dichaete* ♂ × *cinnabar* ♀ ♀, singly

	s D	s	cn D	cn
63 <sup>1</sup>	154	63	158	74
64	136	126	50	67
65	82	82	66	101
66	35	38	40	32
67	73	50	29	26
Totals.....	326	296	185	226

<sup>1</sup> This culture was not included in the totals. The results indicate segregation for a III-chromosome recessive lethal.

TABLE 2  
*B.C.*, *star*  $\left(\frac{s}{cn}\right)$  ♀ ♀ × *cinnabar* ♂ ♂, singly

	s	cn	s cn	-
73	76	72	44	44
75	80	91	47	62
76 <sup>1</sup>	55	54	27	19
77	147	153	104	98
84	96	93	71	75
Totals.....	399	409	266	279

<sup>1</sup> The results of culture no. 76 were not included in the totals, because they differ so widely from those of the other four cultures.

age survival value of vestigial and the low recombination per cent for black and vestigial. They do not appear to be connected with each other as subsequent tests have shown.

The low viability of vestigial has given trouble heretofore when cultural conditions were not of the best, or when care was not taken to run the culture the full period or to shake out all

vestigial flies from the culture bottles. The cultures reported in this paper up to this point were conducted by the two-day vial method described in a previous paper (Clausen and Col-

TABLE 3  
*P*<sub>1</sub>, cinnabar vestigial ♀ × black ♂; *B.C.*, *F*<sub>1</sub> wild-type ♀♀ × black cinnabar vestigial ♂♂, singly

	b	cn vg	+	b cn vg	cn	b vg	b cn	vg
286	212	103	12	7	12	9	—	—
287	277	126	19	5	30	16	—	1
288	266	165	15	14	25	24	1	—
290	132	57	5	8	8	10	—	—
291	127	49	2	4	14	11	1	—
292	179	114	12	3	13	11	—	—
293	202	127	8	3	19	19	—	1
294	269	125	9	11	26	13	—	—
295	173	86	13	4	11	13	1	—
296	280	137	13	4	16	16	3	1
Totals...	2117	1089	108	63	174	142	6	3

TABLE 4  
*P*<sub>1</sub>, black cinnabar ♀ × vestigial ♂; *B.C.*, *F*<sub>1</sub> wild-type ♀♀ × black cinnabar vestigial ♂♂, singly

	b cn	vg	cn	b vg	+	b cn vg	b	cn vg
328	141	89	8	4	13	4	1	—
329	88	76	7	3	9	5	—	2
330	178	144	11	10	24	11	1	1
333	127	77	3	4	14	4	—	—
334	183	123	11	10	15	9	—	—
335	154	103	9	—	12	3	—	—
337	210	176	9	9	15	14	—	—
339	299	203	13	8	12	12	—	1
380	253	170	14	10	23	14	1	1
381	240	165	17	12	20	12	1	1
Totals...	1873	1326	102	79	157	88	4	6

lins, '22). Since care was taken to run cultures for the full period and no trouble was experienced in shaking out all vestigial flies from these small vials, it seemed likely that the low survival of vestigial was due to unfavorable cultural conditions.

Since vestigial has not exhibited low viability under the new culture methods worked out by Bridges ('21), it seemed wise to make a direct comparative test of the two-day vial method

TABLE 5  
*P<sub>1</sub>, cinnabar ♀ × black vestigial ♂; B.C., F<sub>1</sub> wild-type ♀ × black cinnabar vestigial ♂♂, singly*

	cn	b vg	cn	vg	b	cn vg	+	b cn vg
382	257	223	7	5	33	25	1	4
383	240	154	12	6	19	14	2	2
384	238	157	9	6	31	15	—	—
385	246	178	12	7	22	8	2	1
390	242	184	12	5	21	15	1	1
391	239	178	14	5	27	8	—	1
392	210	188	13	12	22	19	1	—
393	217	166	7	5	21	10	1	—
401	226	154	16	4	25	9	—	1
402	253	227	21	8	15	24	2	3
Totals...	2368	1809	123	63	236	147	10	13

TABLE 6  
*P<sub>1</sub>, wild-type ♀ × black cinnabar vestigial ♂; B.C., F<sub>1</sub> wild-type ♀ × black cinnabar vestigial ♂♂, singly*

	+	b cn vg	b	cn vg	b cn	vg	cn	b vg
386	291	93	13	6	24	15	—	1
388	269	99	25	11	24	12	1	3
394	320	126	21	10	41	13	1	—
395	283	172	15	15	40	28	1	2
396	256	96	16	8	36	18	1	2
397	270	167	28	9	30	29	—	3
398	219	79	13	4	17	9	—	3
399	220	80	18	4	25	10	—	—
400	342	219	25	19	34	15	—	4
409	180	76	15	4	15	9	—	1
Totals...	2650	1201	189	90	286	158	4	19

with his half-pint banana-agar method. For this purpose three wild-type *F<sub>1</sub>* females from wild-type ♀ by black cinnabar vestigial ♂ were mated to black cinnabar vestigial males and progeny reared by the vial method in comparison with progeny

from three similar pairs, made up with sister females, reared by the banana-agar method. The data are contained in table 8. The results indicate clearly that the low survival of vestigial in the two-day vials was due to unfavorable cultural conditions which resulted in differential elimination of vestigial flies. In the half-pint bottles with banana-agar medium the vestigial classes contained 46.1 per cent of the total number of flies. In the two-day vials only 22.6 per cent of the flies were of vestigial classes, a lower percentage than that obtained in any of the previous back-cross cultures. The recombination percentages are not, however, significantly changed by the cultural conditions, as shown by calculations from these data, as follows:

	b-en	en-vg	b-vg
Half-pints.....	6.1	8.9	14.8
2-day vials.....	5.1	9.7	13.6

In view of the variations which are known in recombination percentages it is doubtful that the differences have any significance. If the low viability is dependent exclusively on vestigial, no change in recombination percentages is to be expected.

The average b-vg recombination per cent from the data in tables 7 and 8 is 13.3 per cent, a significantly lower value than that given by Bridges and Morgan ('19), 17.8 per cent, as the result of their more extensive investigations. This is very unfortunate because it leaves the exact position of cinnabar still in doubt. The data of each of the experiments appear to be homogeneous; and, while the results of the four experiments appear to be somewhat different, it is believed that these differences are not as great as their common difference from standard data. If we use the direct b-en value of 5.3 the probable locus of en would be 51.8, which lies to the left of purple. If, however, we correct the values so that the b-vg value = 18.5 (the map distance from b to vg determined by Bridges and Morgan) the value for b-en becomes 7.0 which gives a locus at 53.5, a position somewhat to the right of purple. Since purple has been used so

extensively in work on the second chromosome, it is important to know the relation between purple and cinnabar, before the latter can be employed satisfactorily in second-group studies.

TABLE 7

*The four complementary back-cross experiments giving data on the relations of black, cinnabar, and vestigial. A summary of the data included in tables 3 to 6 with the recombination percentages*

TYPE	—	+-	-+	++	TOTALS	b-cn	cn-vg	b-vg
b— cn vg	3206	171	316	9	3702	4.9	8.8	13.2
b—cn vg	3199	172	245	10	3626	5.0	7.0	11.5
—cn b vg	4177	186	383	23	4769	4.4	8.5	11.9
b—cn vg	3851	279	444	23	4597	6.6	10.2	15.7
Totals....	14433	808	1388	65	16694	5.2	8.7	13.2

TABLE 8

*Comparison of half-pint and two-day vial methods of culture.  $P_1$ , wild-type ♂ × black cinnabar vestigial ♀; B.C.,  $F_1$  wild-type ♂ × black cinnabar vestigial ♀, singly*

		b-cn-vg	b	cn-vg	b-cn	vg	cn	b-vg
460	95	98	12	9	9	6	—	1
461	176	145	6	11	14	16	—	—
462	176	148	16	5	27	15	—	—
Totals half-pints.....	447	391	34	25	50	37	—	1
463	168	51	4	—	19	6	—	—
464	232	54	15	8	21	8	2	1
465	241	75	14	2	25	8	—	3
Totals 2-day vials.....	641	180	33	10	65	22	2	4

## RELATION BETWEEN PURPLE AND CINNABAR

By both methods of calculation the loci of *pr* and *cn* cannot be far apart. The position at the right of *pr* seemed the most logical one and further operations were based upon this assumption. Since the loci must be close together, it was desirable to devise a method of obtaining the *pr-cn* combination which would reduce somewhat the number of individuals to be tested.

At that time we had on hand stocks of black cinnabar and jaunty purple flies. The method adopted for obtaining the desired combinations was to test cinnabar males of  $F_2$  from an original cross of jaunty purple ♀ by black cinnabar ♂ by mating them to virgin wild-type females from the same cultures. Considering only single crossing-over and an arrangement of factors of the type,  $\frac{b}{j} \frac{cn}{pr}$  the  $F_2$  cinnabar males might be of

three genotypes; viz.,  $\frac{b}{cn}$ ,  $\frac{b}{j} \frac{cn}{cn}$ , and  $\frac{b}{j pr} \frac{cn}{cn}$ .

The first type should be very rare and may safely be neglected, since its production involves crossing-over between *b* and *j*, which are only 0.2 of a unit apart. The third combination is the one desired, and its ratio to the second type should be 0.8:6.0, assuming correctness of the calculations of the locus of *cn*, or about one cinnabar in eight should be of the desired constitution. The mating of  $F_2$  cinnabar males to sister  $F_2$  wild-type females should furnish diagnostic progeny, if the wild-type females chosen were  $\frac{b}{j pr} \frac{cn}{cn}$ , for then  $\frac{b}{j} \frac{cn}{cn}$  males would give jaunty flies with wild-type eye-color for the most part, whilst  $\frac{b}{j pr} \frac{cn}{cn}$  would yield jaunty purple flies, and, by crossing-over, a few jaunty purple cinnabar flies. No counts were made of the flies in these cultures, but after a number of trials a few jaunty purple cinnabar flies were obtained in culture no. 564, and from these a jaunty purple cinnabar stock was established.

The purple cinnabar flies thus secured were readily recognizable, the difference between the wild-type and purple eye-

colors proving less easy of recognition than that between cinnabar and purple cinnabar. This interesting observation is in line with the disproportionate modification of vermilion by purple noted by Bridges ('19). The four eye-color types, wild-type, purple, cinnabar, and purple cinnabar, therefore, gave no difficulties in classification beyond that noted by Bridges ('19) for purple as contrasted with wild-type.

In order to determine the locus of *en*, cultures were immediately established of wild-type ♀ by jaunty purple cinnabar ♂. Ten cultures of *F*<sub>1</sub> wild-type ♀♀ by jaunty purple cinnabar ♂♂ were established, all of which were successful. The results from them are recorded in table 9. The recombination percentages based on the totals of this table are as follows; *j*-*pr*, 7.7; *pr*-*en*, 3.6; *j*-*en*, 10.8. These values are in excess of expectation, for *j* is assigned a locus at 46.7, which gives a map-distance of 6.0 for *j*-*pr*. Proportional reductions of the values to this scale would give a *pr*-*en* value of 2.8 and a locus at 55.5, which probably represents the closest approximation to its position from the data thus far secured. Its locus, therefore, is evidently to the right of *pr*, and the provisional locus at 51.± assigned to it in the latest map of *Drosophila melanogaster*, which was based upon the only data available at that time, viz., the results of the complementary *b*-*en*-*vg* back-crosses, is in error (Morgan, Sturtevant, and Bridges, '22).

#### THE LOCUS OF JAUNTY

The exact locus of *j* has never been determined. The locus provisionally assigned to it at 46.7, 0.2 of a unit to the right of *b*, is based on a single recombination between *b* and *j* obtained in 462 flies by Muller ('16). Other attempts to secure combinations of black and jaunty failed (Bridges and Morgan, '19, p. 163), but left no doubt that *j* must be close to *b*. The question of its precise location is not an important one, in view of the excellence of black as a representative of this region of the chromosome, but we became interested in the problem of securing a black jaunty combination, and eventually did obtain it.

The method employed was similar to that used for securing the cinnabar purple combination. From *F*<sub>2</sub> of black cinnabar

♀ by jaunty purple ♂, black males were isolated and tested in mass cultures by mating with jaunty females. In order to keep the  $\frac{b}{j} \frac{cn}{pr}$  segregation going, parallel cultures were estab-

TABLE 9

*P<sub>1</sub>, wild-type ♀ × jaunty purple cinnabar ♂; B.C., F<sub>1</sub> wild-type ♀ ♀ × jaunty purple cinnabar ♂ ♂, singly*

	+	j pr cn	j	pr cn	cn	j pr	pr	j cn
585	143	91	6	12	2	9	—	—
586	196	160	13	18	9	5	—	1
587	153	147	11	16	4	5	—	—
588	195	167	16	16	9	8	1	—
589	174	116	10	11	5	7	2	—
590	147	88	15	4	4	2	—	—
591	162	132	11	10	10	3	1	—
592	177	123	11	13	6	5	1	—
593	179	173	21	15	3	5	—	—
594	141	112	10	10	6	5	—	1
Totals...	1667	1309	124	125	58	54	5	2

TABLE 10

*P<sub>1</sub>, jaunty cinnabar ♀ × black purple curved ♂; B.C., F<sub>1</sub> wild-type ♀ ♀ × black jaunty purple ♂ ♂, singly*

	b pr	j	b j	pr	b	j pr
706	242	238	—	2	18	16
707	253	253	—	—	14	8
708	241	246	1	—	11	15
709	250	289	1	1	17	17
710	260	283	1	—	15	20
711	220	252	—	—	14	16
712	266	246	—	3	19	14
713	247	256	—	—	20	13
715	275	242	—	—	15	13
Totals...	2254	2305	3	6	143	132

lished by mating wild-type flies from the same cultures inter se in single pairs, the nature of their progeny indicating clearly whether or not the wild-type flies were of the desired constitution. At the same time jaunty male segregants were also isolated and mated to black females in order to guard against the

possibility that *j* was to the left of *b* instead of to the right. Tests of the jaunty males did not yield the desired combination, but eventually one of the mass cultures of jaunty ♀♀ by black ♂♂ gave some jaunty offspring, which, when inbred, culture no. 649, produced black jaunty purple flies. One hundred and seventeen black males were tested before the desired combination was secured, which in itself indicated a locus very close to *b*.

With the establishment of the black jaunty stock the determination of the locus of *j* became purely a routine task. *F*<sub>1</sub> was obtained by mating jaunty cinnabar ♀ with black purple curved ♂. For the back-cross ten cultures of *F*<sub>1</sub> wild-type ♀♀ by black jaunty purple ♂♂ were established, nine of which proved successful. The data are recorded in table 10. No double cross-overs were secured, consequently columns for black jaunty purple and wild-type flies are omitted from the table. The recombination per cents secured are as follows: *b-j*, 0.2; *j-pr*, 5.7; *b-pr*, 5.9. The *b-pr* value of 5.9 is only 0.3 of a unit less than the map-distance given by Morgan and Bridges ('19), so that no correction of the *b-j* value is necessary. These data confirm the provisional location of *j* at 46.7. At the present time stocks of black jaunty purple and black jaunty cinnabar are on hand, as well as other combinations of these characters, so that they are available for any experiments in which they may be useful.

#### DISCUSSION OF RESULTS

It is apparent, as pointed out, that the recombination per cents of the different experiments are slightly but significantly different, so that a summary of these not strictly comparable data is not given. The reason for the discrepancies is not obvious, but attention should be called to the fact that the stocks employed were obtained from the Columbia laboratories several years prior to their use in these experiments. They have had a rather long independent history during which they have been used in a variety of ways, which might have given opportunity for the origination or the incorporation in them of genetic differences in crossing-over similar to those which have been described in other

stocks by many investigators. Speculation without further experimentation cannot be expected to lead to an interpretation of these difficulties. I have been interested in a limited problem, namely, the determination of the locus of *cn*, and incidentally that of *j*, accurately enough so that cinnabar may be employed henceforth in genetic work. This problem has been solved by a determination of its linear order in relation to the most used genes lying near it in the series. Its subsequent employment will lead to the collection of additional data which may be used in correcting the particular values which I have obtained.

## SUMMARY

1. Cinnabar, symbol *cn*, is a new eye-color identical in appearance with vermilion and scarlet.
2. The locus of *cn* lies to the right of purple at approximately 55.5 in the second chromosome.
3. Purple is a disproportionate modifier of cinnabar, analogous in its effect on this character to that on vermilion.
4. The provisional locus of *j* at 46.7 is confirmed by back-cross determinations.

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## REACTIONS OF HYDRA TO CHLORETONE

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FIVE FIGURES

Chloretone is a useful narcotic to employ when one attempts to analyze the reaction complexes of Hydra. This reagent lends itself well to such efforts since it is a specific poison for neurones. A Hydra, therefore, under the influence of weak solutions of this drug loses the power to operate those muscles that are stimulated only indirectly through nervous tissue, while those muscles that are stimulated directly retain their power to contract. Aqueous solutions of chloretone have been employed in this work. Sometimes eosin has been added to give color to the solution; at other times magnesium sulphate or India ink were mixed with it.

We have studied the reactions of *Hydra viridis*, *Hydra grisea*, and *Hydra fusca*. Fresh material was usually employed. However, specimens taken from well balanced aquaria that had been in the laboratory for weeks showed no departure in their reactions from those of the freshly collected polyps.

The polyp of Hydra has a body proper, which is divided into basal disc, stalk, 'stomach' (or distal two-thirds) and peristome (fig. 3). A zone of tentacles arise from about the base of the peristome. The tentacles are the most active parts of the polyp. And yet the body seems to be the dominating region. for Schultz ('13) observed that during periods of starvation the tentacles undergo a process of autodigestion. The outcome of this autodigestion, if starvation be protracted enough, is the complete disappearance of the tentacles and even peristome, while the body is saved.

A conspicuous feature of the histology of the Hydra's peristome seems to have escaped the attention of earlier investigators, i.e., its endoderm contains an additional type of secreting cells.

These cells resemble mucous cells, and differ from the secreting cells to which Tannreuther ('09) refers when he writes that "many of the endodermal cells in the distal half of the Hydra have a glandular appearance and are most active in the secretion of the digestive fluid which aids in the breaking up of the food in the enteron." These conspicuous cells of the peristomal endoderm differ from the other glandular cells in two ways: a) their cytoplasmic contents are more uniform in size and shape and stain less deeply in the basic dyes; and b) their secretion droplets have a dark-brown appearance when seen by transmitted light in a living specimen. After an animal has made an effort to ingest a mass of food these cells lose some of their color. It appears, therefore, that the secretion of these peculiar cells plays a rôle during the ingestion of food (figs. 4 and 5).

It is possible that the secretion of these peristomal endodermal cells takes part in paralyzing prey without the aid of nematocysts. Wagner ('04) saw such paralysis but he referred it to the tentacles saying, "Apparently this (paralysis) is sometimes brought about by some purely fluid discharge from the tentacles."

We have nothing to add further to the above account of the histological details of Hydra. We must, however, call attention to the fact that Hadži ('09) has demonstrated a nerve net associated with the ectodermal epitheliomuscular tissue, while the endodermal tissue is supplied with but isolated neural elements, if any be present therein.

The contraction of the ectodermal myonemes, which are disposed parallel to the axis of the body, results in a shortening of the body. The contraction of the endodermal myonemes, which lie at right angles to the axis of the body, causes the polyp to lengthen. When we keep in mind that the conspicuous nerve supply is in the ectoderm and the meagre one (if any) is in the endoderm, it becomes interesting to observe how rapidly a Hydra may contract as over against its rate of elongating. In the first case we have an example of a neuromuscular function, while in the latter we have an incomplete neuromuscular function. In addition to this frequent contraction and elongation, Hase ('10) found two types of 'Bewegungsform' (pulsation) in Hydra. One type of

pulsation is sudden which by an expansion before and a contraction behind an ingested body suddenly shifts it from one region of the body to another. The second type of pulsation that Hase observed was similar to the neuroid waves that pass over a sponge's body when it is strongly stimulated. This pulsation is sustained for from fifteen to thirty minutes in travelling over the length of the body. In Hase's first type of pulsation it appears that the ectoderm and endoderm are cooperating, while in the latter it may be that only

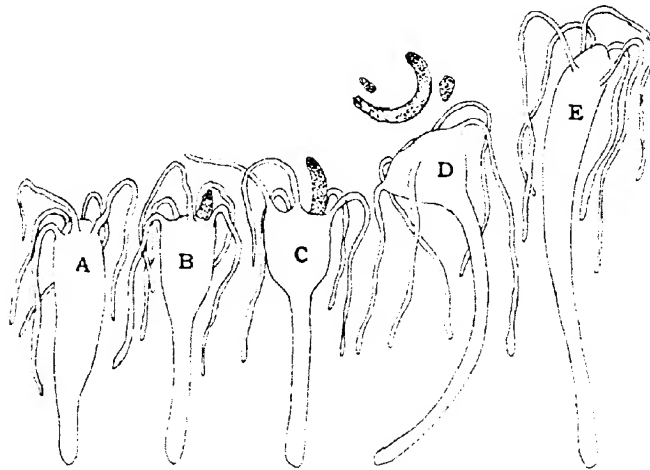


Fig. 1 A shows contour of specimen after having been injected with chloretone and rinsed in two changes of water. B, C, and D show the advance of the peristaltic wave. E shows the contour of the animal after this neuroid wave of peristalsis had spent itself.

the endoderm is involved because of the extremely sluggish peristalsis. Wagner ('04) has seen ejection of food "performed several times, and in every case the food was forced out by a sudden squirt that threw the debris to some distance" (p. 611). He records no slow peristalsis. We have seen that when an indifferent substance like India ink has been injected into the enteric cavity of a Hydra no rapid peristalsis arises but a slow wave carries the foreign material towards the oral end and even-

tually delivers it through the mouth. For two hours after a droplet of India ink has been injected into a Hydra's body, the ink was slowly carried towards the mouth by a constriction that travelled behind it as a very slow wave. When the ink gets near the bases of the tentacles an interesting process has been observed. This is the operation of a sphincter which lies at the base of each tentacle. When a droplet of ink had reached the relative position indicated at G, figure 2, the sphincters closed so as to occlude the lumina at the bases of the tentacles. These sphincters, remaining closed, directed the ink, as it was slowly advanced by the body wave, to the mouth. After the ink was thrown out as shown in figure 2, I, the sphincters again opened. A similar observation has been made when a bubble of air was employed instead of the ink. On one occasion a Hydra, just taken from the pond, was slowly ejecting material from the enteron. This specimen showed closed sphincters most clearly as the material was passing by the bases of the tentacles.

Finally these sphincters may be demonstrated in another manner. If some harmful material be injected into the enteron, the body can be greatly distended as though it were a thin-walled rubber sac and yet the tentacles will not be inflated. We have frequently inflated Hydras' bodies thus and in no case have we succeeded in distending the tentacles. Thus both by observation and experiment, sphincters at the bases of tentacles have been demonstrated.

An interesting character of these sphincters is that they are only under the control of the body proper and do not respond to stimuli arising in the tentacles. This is shown by the fact that when 1 per cent chloretone is injected into the tentacle, the body is always distended. This distention of the body through the tentacle may be so great as to widen the base and shorten the axis of a bud. But in these cases, where a body and its bud are thus greatly distended by pressure of a fluid coming in from one tentacle, the sphincters of the other tentacles function; for no other tentacles are inflated. We have here a sharp contrast between the conduct of a tentacle reacting to a stimulus that comes from the body proper and the reaction of a tentacle to stim-

uli arising in it. The sphincter, for example, will prevent a foreign substance passing from the body into the tentacle, while it will not prevent such material entering the body from the tentacle. It becomes evident, therefore, that the Hydra's body dominates the tentacle. Unlike von Uexküll's echinoderms, the ambulacral

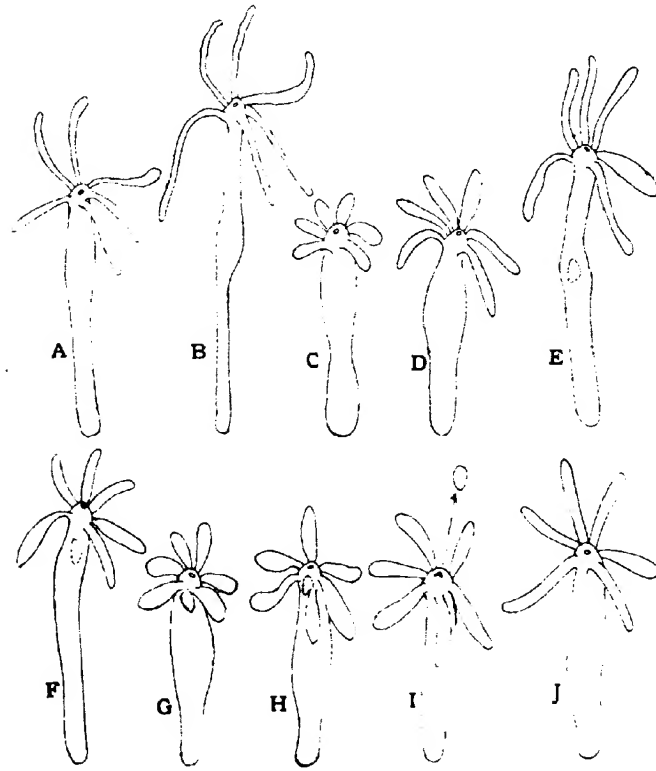


Fig. 2. A and B show the advance of a neuroid peristalsis behind a droplet of India ink. At C and D a contraction had taken place but no constriction of bases of tentacles was evident. E, the neuroid action had carried the droplet of India ink orally. In G and H the ink being near the bases of the tentacles, the bases of the tentacles are seen to be constricted due to the closing of their sphincters. I and J show the sphincters open.

feet of which move the body instead of the body moving the feet, here the body dominates the tentacles.

Through these sphincters the body proper protects the tentacles from harmful substances that may be taken into the enteron and perhaps controls the food supply also of the tentacles.

Having discovered the presence of these sphincters, we were in a position to determine to what extent distribution of absorbed substances is carried on in *Hydra*. It seems that the material absorbed by the endoderm is not widely distributed. For example, we have frequently inflated the enteron with 1 per cent chloretone and in every case the body proper was quickly quieted while the tentacles plied about as actively, if not more so, as they had done under normal conditions. *Hydra*, therefore, must have nothing comparable to a circulatory medium. Even the mesoglea is not a path of wide diffusion. We can understand, thereby, why it is, as Tannreuther ('09) observed, that at a region where a gonad or a bud is to be formed, a special accumulation of food must be made. These most active regions, because of the lack of a circulatory medium, must be further taxed by digesting and absorbing a special and increased food supply. A disadvantage of the diploblastic character of *Hydra* thus becomes apparent.

The peristomal endoderm seems to be able to reject chloretone. The base of the peristome may be constricted so as to occlude the lumen and cause the free ends of the peristomal endodermal cells to interlock about the axis of the peristomal cone. In this manner the rejection of chloretone on the part of the peristomal endodermal cells may be effected. For the fact has been noted many times that when a *Hydra* is narcotized through injection of chloretone into the enteron, the peristome becomes very active as though it has been cut off from the inhibitory control of the body proper. Under such condition the peristome displays exploratory movements suggesting the lipping of a horse in seeking to grasp a tuft of grass. Here again, the body proper being paralyzed while the intimately anchored peristome is not, is demonstrated the local or restricted manner in which diffusion takes place. No chloretone has been transferred from the body proper to the occluded peristome.

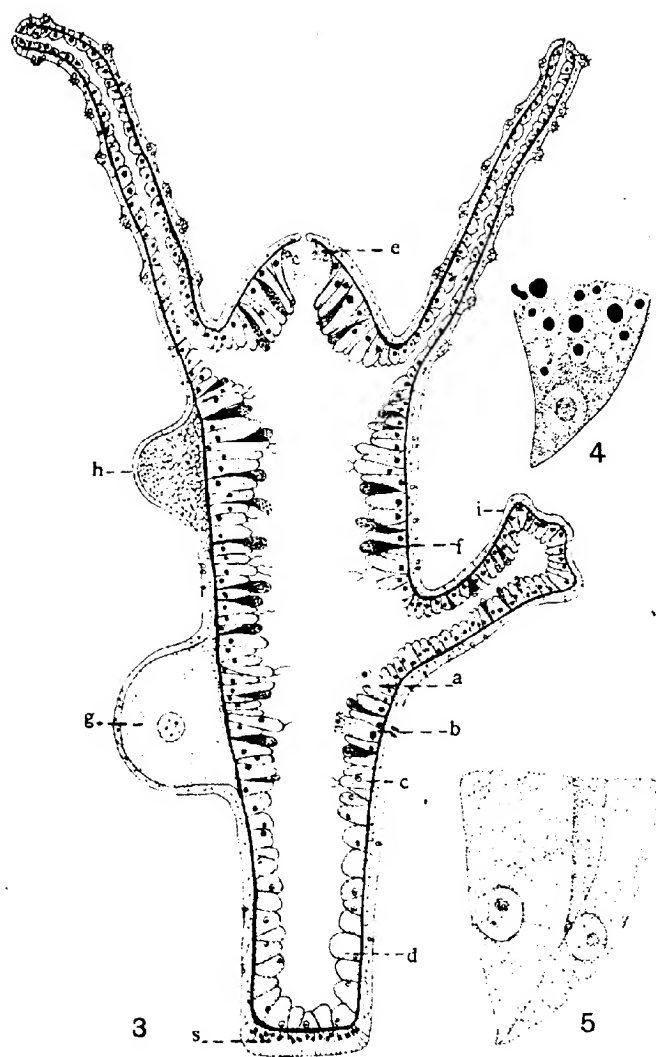


Fig. 3 Diagram of longitudinal section of Hydra. a, epithelio-muscular cell of endoderm with a food vacuole; b, epithelio-muscular cell of endoderm ingesting food particle; c, epithelio-muscular cell of endoderm bearing two flagella; d, epithelio-muscular cell of endoderm of stalk (these are usually more highly vacuolated than those of the oral two-thirds of body); e, peristomal secreting cell (fig. 5); f, secreting cell of endoderm (not found in stalk; fig. 4); g, ovary; h, testis; i, bud; and s, adhesive cells of basal disc.

Fig. 4 Secreting cell of general endoderm (not found in stalk.  $\times 1500$ .

Fig. 5 Peristomal secreting cell (e, fig. 3).  $\times 1500$ .

That we are not dealing with regions of the Hydra's body that are immune to chloretone is shown by the fact that by placing a Hydra in chloretone the tentacles and peristome are quite narcotized. The results obtained through injection, therefore, indicate that tentacles and peristome are not influenced because absorption and diffusion do not carry the chloretone to the closed peristome nor do they carry the narcotic to the tentacles when the sphincters operate.

The injection of chloretone into the enteron soon narcotizes the neuromuscular apparatus of the ectoderm, so that soon after such injection the body elongates and remains elongated, as it does when the specimens have been immersed in solutions of chloretone. In such narcotized specimens no sudden contraction of the body is possible, though the tentacles and peristome be freely plying to and fro. However, the neuroid peristalsis, such as Hase observed and such as we have recorded above when Hydra is egesting some inert material, like India ink or air, may yet be maintained. The following observation selected as the most conspicuous example of its kind will serve as an illustration. A *Hydra fusca* was injected with 1 per cent chloretone at 10.37 a.m. near the base of its body. Immediately after the operation it was rinsed in two changes of spring water. The specimen after rinsing had the appearance of A, figure 1. Here the basal portion was constricted. This constriction had crowded beneath a mass of enclosed food. No advance of this constricted region could be noticed, as the specimen was being watched; but at 10.40 a.m. it was evident that such advance was taking place, for the constricted region had become longer and the distended region shorter while an ingested worm was being crowded from the mouth (fig. 1, B). At 10.42 a.m. further advances of the neuroid peristalsis could be observed (fig. 1, C). At 10.47 a.m. the neuroid wave had forced the food, without a spurt, from the mouth and was spending itself at the end of the body resulting in the spreading apart of the bases of the tentacles (fig. 1, D). By 10.48 a.m. the specimen had assumed an elongated form (fig. 1, E), in which condition it lay until 10.55 a.m., when it assumed the normal shape. Thus a wave, in passing from the basal third

of the body to spend itself at the oral extremity, consumed a period of ten minutes. The chloretone had put the neuromusculature of the ectoderm out of function and the endoderm behaved as a neuroid tissue. The tardiness of this peristalsis does not seem to be due to the food-mass against which it had to operate, for we have observed similar waves advancing from the wounded region to oral end of green, brown, and gray Hydras that has been similarly injected with chloretone though they had in them no large mass of food.

A similar result may be obtained when a Hydra is placed in a 0.33 $\frac{1}{3}$  per cent solution of chloretone and then injected with 0.5 per cent solution of chloretone, thus subjecting both the ectoderm and endoderm to the narcotic directly.

In all instances after injection with chloretone, the animals would contract and remain contracted for a brief period until the chloretone had time to operate on the ectoderm. This preliminary contraction would sometimes complicate the observations when it came to determining the exact position of the neuroid wave and its progress. To obviate this difficulty, a green Hydra was placed in 0.33 $\frac{1}{3}$  per cent chloretone. Green Hydras in this percentage of chloretone gradually elongate. After the process of elongation has set in, the animals cannot be caused to contract by mechanical stimuli. However, after the animals have thus remained elongated in 0.33 $\frac{1}{3}$  per cent chloretone for an hour, they appear to have adjusted themselves to this strength of chloretone and gradually regain the use of their ectodermal neuromusculature. If now the solution be made stronger, the green Hydras will elongate again.

We took advantage of this characteristic of the green Hydras to observe a neuroid wave. After a green Hydra lying in 0.33 $\frac{1}{3}$  per cent chloretone had elongated, it was injected with 0.5 per cent chloretone; a distended region appeared on the body at the point of the injection. Direct observation did not show that this distended region was moving as a slow peristaltic wave. However, a discharged nematocyst was lying at the lower (aboral) base of the distended region. By fixing the attention on this nematocyst it was seen that there was in reality a peristaltic

wave that traveled towards the mouth leaving the attached nematocyst behind. After an hour the wave had advanced but a short distance. The Hydra now began to move to and fro and a drop of 1 per cent chloretone was added. This caused it again to elongate. Upon the elongated animal two waves appeared. In this case the position of one of the waves could be determined by a ragged mass of cells that lay on its lower (aboral) slope. Before the animal had again taken to moving to and fro, this wave had passed away from this stationary mass of cells. Thus with chloretone operating both within and without the specimen, two neuroid waves were observed which had moved very slowly towards the mouth.

Green Hydras do not react differently from other Hydras to 1 per cent chloretone that has been injected into the enteron; but a conspicuous difference is seen when these two kinds of Hydras are put side by side in 0.33 $\frac{1}{3}$  per cent chloretone. Here we have found after repeated trials, that the green Hydras elongate and can eventually recover their activity if kept in the chloretone solution, while *Hydra fusca* or *Hydra grisea* contracts so severely that within ten to twenty minutes it has squeezed out of its mouth a mass of its endoderm. When contraction has advanced so far that the endoderm extrudes, the specimen cannot be revived. After many observations we have seen that *Hydra viridis* elongates and in time adjusts itself to being immersed in 0.33 $\frac{1}{3}$  per cent chloretone while the *Hydra fusca* and *Hydra grisea* contract as though convulsed and are killed within one-half hour or less.

Before further considering this reaction of green Hydras, as over against brown and gray Hydras, to chloretone, it should be pointed out that the distinction is less conspicuous when solutions of less concentration of chloretone are employed. For example, green Hydras are scarcely affected by 0.1 per cent chloretone, while the brown and gray ones, in this 0.1 per cent solution, slowly elongate and in time become accustomed to the poison as did the green Hydras to 0.33 $\frac{1}{3}$  per cent chloretone.

In seeking for an explanation of this difference, we naturally turned to the presence of the zoochlorellae of *Hydra viridis*. It

occurred to us that the oxygen given off by the photosynthetic organisms might account for the difference in the effect of chloretone on Hydra as over against its effect on *Hydra fusca* and *Hydra grisea*. Accordingly freshly collected Hydras of the three species were placed in the dark for 24, 48, 72, and 120 hours. After thus treated, each lot of the brown and gray Hydras contracted convulsively when placed in 0.33 $\frac{1}{3}$  per cent chloretone; while each lot of the green ones on being placed into 0.33 $\frac{1}{3}$  per cent chloretone after having been in the dark would at first elongate, but within several minutes they would shorten. This shortening would continue until the colorless ectoderm, in great part, would be crowded at the basal end of the rounded mass, leaving only a very thin layer of ectoderm to enclose the endoderm that was lying on the oral side of the mass. The green specimens remained in this position until transferred to spring water. When thus transferred to spring water these dark exposed green Hydras recovered. It has thus been demonstrated that exposure to dark does not change the reactions of gray and brown Hydras to 0.33 $\frac{1}{3}$  per cent chloretone but does cause the green ones to react more like brown and gray ones. It is thus suggested that the oxygen product by photosynthesis in the algal symbiont is appropriated by the Hydra.

In one instance we found a green Hydra that has a ciliated parasite, that was about 200 $\mu$  long, anchored to its endoderm. When this specimen was placed in chloretone, the parasite, that had remained quiet up to this point, became very active at once. It remained quiet for twelve minutes when it was egested by the Hydra. This *Stenostoma*-like parasite lived two minutes later or after the parasitized Hydra had been in the 0.33 $\frac{1}{3}$  per cent chloretone fifteen minutes. This green Hydra contracted so severely as to evert its endoderm as the brown and gray Hydras do when placed in 0.33 $\frac{1}{3}$  per cent chloretone. This is the only example we have observed in which a green Hydra reacted to immersion in 0.33 $\frac{1}{3}$  per cent chloretone as do brown and gray ones. This may have been due to the amount of oxygen taken up by the parasite.

## SUMMARY

In reacting to chloretone the ectoderm behaves as a neuromuscular tissue; while the endoderm functions as a neuroid tissue.

A sphincter has been observed at the base of each tentacle. This sphincter operates in preventing food from passing from the enteron into the tentacle but does not prevent passage of material from the tentacle into the enteron.

There is no extensive diffusion of absorbed chloretone through the tissues of the body. A diploblastic animal, therefore, cannot possess anything comparable to a circulatory medium.

Green Hydras under ordinary conditions withstand the effects of chloretone much more than do brown and gray ones. Our experiments indicate that the presence of zoochlorellae in *Hydra viridis* are responsible for its resistance to chloretone, for we can greatly lower this resistance by placing the green *Hydra* in the dark. This lends weight to the contention that the algae of the green *Hydra*'s endoderm are symbionts.

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## FURTHER STUDIES ON INHERITANCE OF EYE DEFECTS INDUCED IN RABBITS

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FOUR TEXT FIGURES AND ONE PLATE (FOUR FIGURES)

In continuation of our studies on the production and transmission of eye defects in rabbits (Guyer and Smith, '20) we have carried on through several generations a new strain which had just been secured when our second study went to press. We have also made attempts to secure eye defects by the injection of pulped rabbit lens directly into rabbits themselves. Our single success in this endeavor is discussed in the latter part of this paper.

The new strain of defective-eyed rabbits referred to centers in a female 16A1, from a stock wholly unrelated to the line in which we had originally produced eye anomalies. She was one of a litter of ten born of a mother which had been shipped in a few days earlier from Minneapolis. She was first mated to a brother 16A2, December 7, 1918, and four normal-eyed young (N16 series), one male and three females, were born January 7, 1919. One of these females died at the age of four months. Female 16A1 was remated to 16A2 on March 5, 1919, and on the twelfth day of pregnancy injections of fowl serum immunized to rabbit lens were begun. The details of this experiment, as well as those connected with a later mating, are given in tables 1 and 2. One of the four young born from this mating had both eyes markedly abnormal (fig. 1). It lived only five weeks. About three months later female 16A1 was mated again, this time to a male, no. 50, just arrived from Chicago. A third treatment with lens immunized serum was begun on the eighth day of pregnancy (table 2). Five young were born July

21, of which two males (70A1 and 70A2) each had both eyes defective (fig. 1). One young died shortly after birth, hence the condition of its eyes was not determined. The remaining two apparently had normal eyes. One of them, a female 70A3, lived to become adult, the other died while young. The eyes of male 70A2, originally microphthalmic, gradually became resorbed as he grew older until scarcely a trace of either eyeball remained. The left was from the very first somewhat collapsed and barely visible; the right was about one-third normal size, clouded, and so badly rotated to the rear that the sight lay partly obscured by the lids at their upper, hinder angle.

From a mating of 70A3, the normal-eyed female, and 70A2, a litter of seven (71A series) was born March 15, 1920. Of these (fig. 1) a male, 71A1, had both eyeballs practically missing; two males, 71A2 and 71A5, had eyes normal in appearance; the fourth male, 71A3, had both eyes very abnormal, one of them showing but a trace of eyeball; a female, 71A4, had the left eye microphthalmic and otherwise defective, and the right eye of questionable normality; another female, 71A6, had an abnormal right eye with cleft iris, silvery hue, and opaque lens; the remaining female, 71A7, had both eyes apparently normal. The defects were of the same general types as those described in our earlier paper ('20) for our 3A1 strain.

A second mating of 70A2 with 70A3 yielded a litter (71B series) of four, two of which, 71B1 and 71B2, each had both eyes defective. Both eyes of the male 71B1 were microphthalmic, with the left eyeball collapsed and all but gone and the right, about half normal size, with some opacity of the lens. The female, on the contrary, had the right eye abnormally enlarged (glaucomatous) and rotated backward, with a caseous-looking lens. The left eye, although of approximately normal size, had a cleft iris and a silvery look. A litter of five (130A series) was secured from mating 71B1 and 71B2 (fig. 1). One died before the condition of the eyes could be determined. Each of the remaining four has both eyes defective. Female 130A2, for example, has a marked distension and protrusion (buphthalmia) of the left eyeball, which is also markedly rotated

backward—so much so, indeed, that only one side of the cornea is visible when the lids are forcibly drawn back at their upper, outer angle (fig. 6). It is an interesting fact that the part of

TABLE I  
*I. Immunization of fowls*

DATE—1919	FOWLS INJECTED	NUMBER OF RABBIT LENSES USED	NORMAL SALT SOLUTION	DOSAGE PER FOWL
			cc.	
February 8	6	6	20	2.0 cc. subcutaneously
February 15	6	8	20	1.0 cc. intravenously
February 22	6	6	15	1.0 cc. intravenously
March 1	6	8	15	1.5 cc. subcutaneously
March 19	2	4	8	3.0 cc. intraperitoneally

*II. Treatment of rabbits*

DATE OF INJECTION	IDENTIFI- CATION NUMBERS OF RABBITS	DAYS PREGNANT	DOSE OF SERUM	REMARKS
			cc.	
March 17	14A5	9	4.0	Mating, 14A5 ♂ × 44 ♀
	18A2	13	4.0	Mating, 18A2 ♂ × 16A2 ♀
	17	13	4.0	Mating, 17 ♂ × 44 ♀
	16A1	12	4.0	Mating, 16A1 ♂ × 16A2 ♀
	14A4	9	4.0	Mating, 14A4 ♂ × 16A2 ♀
March 19	14A5	11	4.0	
	18A2	15	4.0	
	17	15	4.0	
	16A1	14	4.0	
	14A4	11	4.0	
March 21	14A5	13	3.0	
	18A2	17	3.0	
	17	17	3.0	
	16A1	16	3.0	
	16A4	13	3.0	
March 25	14A5	17	2.5	No. 16A1 bore 4 young April 6; 1 had both eyes defective; it died May 11.
	18A2	21	2.5	No. 17 bore 5 young April 5; eyes normal. No. 18A2 aborted 5 young April 1 and died April 9.
	17	21	2.5	Nos. 14A5 and 14A4 had no young.
	16A1	20	2.5	
	14A4	17	2.5	

the ball exposed where the cornea should be normally, has bulged outward and has lost its white appearance, becoming transparent and simulating a cornea. At first the eye was thought to be double. A female, 92D, of the 'direct lens' line (fig. 2), also similarly exhibits this double sighted appearance in each eye. The right eye of 130A2, however, is reduced in size with incomplete iris and lens somewhat opaque. Female 130A3 (fig. 1) has her right eye much reduced in size, and her left

TABLE 2  
*I. Immunization of fowls*

DATE—1919	FOWLS REINJE. TED	NUMBER OF RABBIT LENSES USED	NORMAL SALT SOLUTION	DAMAGE PER FOWL
			cc.	
June 12	2	4	12	3
June 14	2	4	12	3
June 20	2	4	12	5

*II. Treatment of rabbit*

DATE OF INJECTION	IDENTIFI- CATION NUMBERS OF RABBITS	DAYS PREGNANT	DOSE OF SERUM	REMARKS
			cc.	
June 28	16A1	8	4	Five young born July 21. 1 soon died; 2 had very defective eyes; 2 had normal eyes. 16A1 ? was mated to 50 ?.
June 30	16A1	10	4	
July 5	16A1	15	4	
July 7	16A1	17	4	

eye, although smaller than normal, protrudes and has a lens bearing caseous-looking spots. Each eye of the male 130A4 is slightly reduced in size with incomplete iris and silvery look.

Various matings were made among individuals of the 71A series, some of which are shown in figure 1. Since the transmitted defects show no new features it is unnecessary to review the individual cases. A photograph of the right eye of female 73A2 (from parentage 71A6 by 71A3) is shown in figure 5. Each eye is rotated backward so far that only part of the cornea is visible. The lenses, as glimpsed from the side, appear to be caseous or calcareous. The parts of the sclerotic coats exposed anteriorly are bulging and more or less transparent.

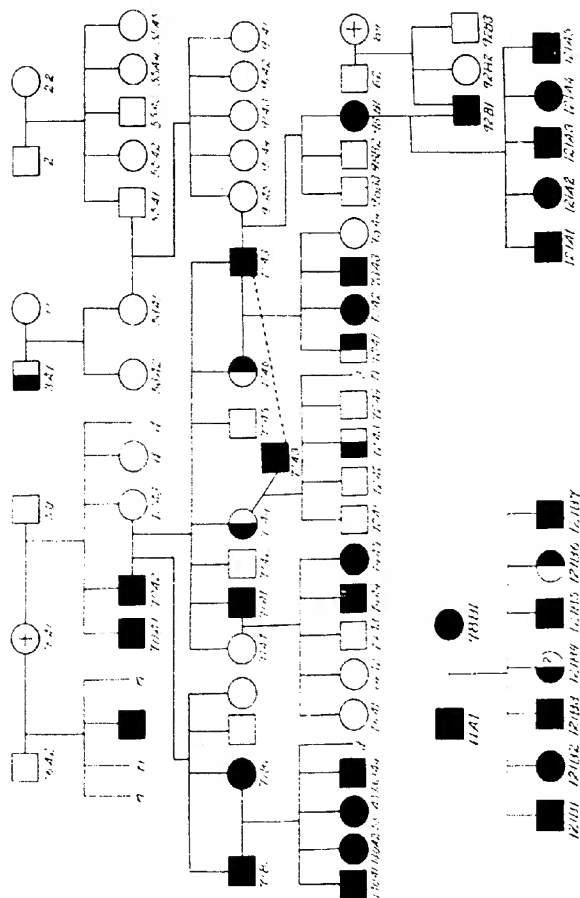


Fig. 1 Chart showing pedigree of some of the defective-eyed individuals. The circle with the + sign in it indicates the female treated with lens-immunized fowl serum (16A1) or directly with pulped rabbit lens (84). The mother of 3A1 was treated with lens-immunized fowl serum. Squares indicate males; circles, females; symbol all black, both eyes defective; right half black, right eye defective; left half black, left eye defective; unshaded, presumably normal-eyed; *d*, died; *n*, normal. Males 16A2, 50 and 2 (upper row) were of normal untreated stock, as were females 11 and 22. Male 62 (fourth row) was of normal untreated stock, while female 84 was injected directly with pulped lens.

In general, a comparison of the pedigree of the 16A2 line (see left side of chart, fig. 1) with our earlier 3A1 strain ('20) shows that the conditions of inheritance are much the same, although more of the defective-eyed individuals had both eyes affected.

By way of controls, sisters of 16A1 were mated to males 16A2 and 50 respectively. Thus 16A3 was mated to male 16A2 March 11, 1919, and bore seven young (C'16A series) a month later, all normal-eyed. This mating was repeated in June and a second normal litter (C'16B series) of five was secured. Upon maturity further inbreeding was practiced. Thus female C'16A2 was mated to C'16A1, female C'16A6 to C'16A7, and female C'16B3 to C'16B4; all of the resulting thirteen young were normal-eyed. Number 16A4, another sister of 16A1, was mated to male 50 and bore eight young, all with normal eyes.

Furthermore, the normal young (N16 series) of female 16A1 and 16A2, secured before she had borne any defective-eyed offspring, were interbred and every one of the eighteen resulting young had normal eyes.

#### DIRECT INJECTION OF LENS INTO RABBITS

While it is of interest to be able to break in on the germ through the use of a foreign serum, it is obvious that the method is a highly artificial one which would not take place under natural conditions. If serological influences are of any considerable importance in the production of germinal changes, they must be such as arise ordinarily in the animal's own blood serum. If its own tissues can on occasion give rise to active substances such as antibodies, then we may have a means, in its own body by which germinal changes may be induced. With this thought in mind we began a series of experiments in which pulped rabbit lens was injected intravenously directly into female rabbits just before or during pregnancy.

In almost all cases the lenses used were from young rabbits from one-fourth to three-fourths grown, because such lenses, when properly ground in a mortar, can be almost completely dissolved or emulsified. According to Jess and Reiss (Jess, '20), in their study of the chemical changes which take place in cataract,  $\alpha$ - and  $\beta$ -crystalline, both soluble in water, make up the greater part of the lens of the young animal. These gradually

decrease in quantity with age, accompanied by sclerosis—a process even more in evidence in cataract lenses. These investigators found from analyses of lens proteins separated from 3,000 beef's eyes that albumoid, the water-insoluble constituent, is poor in valine and alanine, while these two amino-acids are present in considerable quantities in  $\alpha$ - and  $\beta$ -crystalline.

TABLE 3

DATE OF INJECTION 1920	IDENTIFICATION NUMBERS OF RABBITS	NUMBER OF LENSES USED	REMARKS
January 21	S0 and S1	4	S0 had been bred 11 days, and S1 eight days, at time of first injection. No young born.
January 23	S0 and S1	6	
January 26	S0 and S1	6	
January 28	S0 and S1	4	
January 30	S0 and S1	6	
February 2	S0 and S1	4	
February 7	S4	4	S4 bred to ♂ 25 January 28; 7 young born February 28; one died; all had normal eyes. S4 bred to ♂ 4A7, March 27; 6 young born April 27; one ♀2A had defective right eye.
February 9	S4	4	
February 11	S4	4	
February 20	S4	4	
February 20	S0	4	S0 bred February 11; no young. S5 bred February 14 to ♂ 62; 7 young born with normal eyes.
February 23	S0 and S5	6	
February 25	S0 and S5	6	
February 27	S0 and S5	4	
March 1	S0 and S5	4	
March 3	S1, S0 and S5	4	S1 bred February 20; bore 5 young March 20; 3 lived to open eyes; opening of eyes delayed several days; eyes normal.
March 6	S1 and S5	4	
March 8	S1	4	
March 10	S1	4	
March 15	S6	4	S6 bred March 5; 7 young born April 5 but all died before eyes opened.
March 17	S6	4	
March 19	S6	4	
March 22	S6	4	
March 25	S6	4	

In our experiments, after pulping the lenses, sufficient normal salt solution was added to make a dose of from 2 to 4 cc. for each rabbit injected. The injections were made through the marginal vein of the ear. Tables 3, 4, and 5, which illustrate representative treatments, give such technical details as are necessary for a complete understanding of the experiments.

Care was taken (except in the case of one male) to use rabbits which were not of the same strains as those employed in other experiments with lens. In all, eleven different females have been so treated, although since some of them were used on two or even three occasions, this represents twenty-three matings. In one series of experiments (table 4) the male, 62, as well as the females, was injected with the lens emulsion. As with other

TABLE 4

DATE OF INJECTION— 1920	IDENTIFICATION NUMBERS OF RABBITS	NUMBER OF LENSES USED	REMARKS
May 5	80, 81, 85, 86, 21A2, 62	6	62 was a male, the others
May 13	80, 81, 85, 86, 21A2, 62	8	females
May 20	80, 81, 85, 86, 21A2, 62, 84	10	
May 25	80, 81, 85, 86, 21A2, 62, 84	14	
May 28	80, 81, 85, 86, 21A2, 62, 84	14	85 bred to ♂ 53M June 3
June 5	80, 81, 85, 86, 21A2, 62, 84	14	21A2 bred to ♂ 62 June 10
June 12	80, 81, 85, 86, 21A2, 62, 84	14	86 bred to ♂ 62 June 18
June 19	80, 81, 85, 86, 21A2, 62, 84	16	81 bred to ♂ 62 June 19 80 bred to ♂ 62 June 24
June 26	80, 81, 86, 21A2, 62, 84	14	85 bore 6 young July 4; eyes normal
July 3	80, 81, 86, 21A2, 62, 84	12	
July 10	80, 81, 86, 62, 84	10	84 bred to ♂ 62 July 10 21A2 bore 4 young July 11, eyes normal
July 17	80, 84	4	86 bore 3 young July 20, eyes normal 80 bore 4 young July 26, eyes normal 84 bore 5 young August 10; one died, 3 had normal eyes and 1 had markedly defective eyes

experiments in which heavy injections of foreign materials were introduced into the blood stream of females during pregnancy, there was a heavy mortality in the uterine young, consequently the number of young born fell considerably below normal expectations. Of a total of 71 born, 17 died before opening their eyes and were discarded. Of the remaining 54, two individuals, both of the same mother (84) though from different fathers, had defective eyes.

Since the details of representative matings and treatments are given in the tables (3, 4, and 5), it is unnecessary to discuss any of them in the text except that of female 84 which yielded the young with defective eyes.

Female 84 (fig. 2) was the daughter of an albino doe which came in a shipment from Lafayette, Indiana. She was born about a week after the shipment arrived at the laboratory.

TABLE 5

DATE OF INJECTION-1920	IDENTIFICATION NUMBERS OF RABBITS	NUMBER OF LENSES USED	REMARKS
October 20	80, 81, 84, 85, 86 21A2, 53B1	12	53B1 was a male, the others females
October 27	80, 81, 84, 85, 86, 21A2, 53B1	12	
November 3	80, 81, 84, 85, 86 21A2, 53B1	10	
November 10	80, 81, 84, 85, 86 21A2, 53B1	4	21A2 bred to ♂ 53B1, November 15. 21A2 died November 16
November 17	80, 81, 84, 85, 86 53B1	4	81 bred to ♂ 53B1, November 17 86 bred to ♂ 53B1, November 19
November 24	80, 81, 84, 85, 86	4	85 bred to ♂ 53B1, December 2; no young
December 1	80, 81, 84, 85, 86	6	
December 9	80, 81, 84, 85, 86	4	81 bore 2 young, December 17 both died the same day
December 16	80, 84, 85	6	86 bore 5 young, December 20, eyes normal

On January 29, 1920, she was bred to male 25, a black rabbit. On February 7, 9, 11, and 20 respectively she was injected intravenously with pulped rabbit lens, receiving each time 2 cc. of a mixture of an emulsion of four rabbit lenses in normal saline solution. Seven young were born February 28, of which six lived to open their eyes. All of them had normal eyes.

Inasmuch as a series of experiments in the production of spermatotoxins was in progress at this time and considerable quantities of spermatozoa were in demand, 84 was mated to male

made to be of an earlier defective-eyed strain, though he himself had normal eyes. While we have no record of a normal-eyed individual of this strain yielding defective-eyed young in the  $F_1$  generation when crossed into a normal strain and a number of such crosses have been made nevertheless, the experiment as regards this particular mating obviously has to be discarded as inconclusive. It seems possible, however, that the appearance of the anomaly may have been the result of an inherited tendency from the male plus the additional effects of the injected lens, the inherited factors being insufficient in themselves to cause the appearance of the defect.

However this may be, there is no such uncertainty about the next litter which was fathered by a normal male, 62, of a different and normal-eyed strain (fig. 2). In this experiment both the male (62) and the female (84) were injected intravenously with pulped lens, as recorded in table 4. The male received such injections May 5, 17, 20, 25, 28, June 5, 12, 19, 26, and July 3 and 10 respectively. The female was similarly treated except her injections were not begun until May 20 and she received a final injection July 17, 1920. The female, 84, was bred to 62 July 10. She bore five young August 10, of which one died before the condition of its eyes could be determined, three had normal eyes and one, a male (92B1), had markedly defective eyes. Both eyeballs were small, strongly rotated backward, and had chalky-looking lenses. The corneas also were somewhat clouded. As an adult these conditions persisted, although the eyeballs soon began to show indications of collapse.

With these conspicuous defects in evidence in 92B1, we feel that we have secured serologically a specific lens anomaly in a fetal young of a female treated directly with lens material. Male 92B1 (fig. 2) was mated to his sister, 92B2 and a litter of six (105A series) was born September 6, 1921, of which four died before the condition of the eyes could be determined. The remaining two were males, one of which had normal eyes. The other, 105A, has the iris of the left eye cleft and opaque spots in the lens on the right eye.

About two months after she had borne her 92B litter, female 84, without any further injection of lens material, was bred to a male, 53B1, of normal untreated stock. Only one young one, a normal-eyed male, 92C, was born of this mating. When this male became sufficiently mature he bred back to his mother, 84, and five young were born July 6, 1921. Four of these proved to be normal-eyed, but one, a female numbered 92D, has both eyes markedly defective. Each has such a marked backward rotation that the cornea is visible only when the eyelids are drawn back at the outer corner. In each eye the exposed sclera in front has bulged and become transparent, simulating a cornea. This anomaly gives the rabbit the appearance of having a double

eye on each side. The eye is so badly rotated that the condition of the lens could not be determined.

It is an interesting fact that although female 84 received no injection of lens material after her treatment previous to the birth of the 92B series (fig. 2), nevertheless when mated to a normal male (53B1) of normal stock she evidently transmitted something in an unexpressed condition to the young male (92C), for when this male mated back to her, the defective-eyed 92D female just described was produced. Such a result inclines one to the view that the earlier injections of lens into 84 may have led to the formation of antibodies which had acted directly on the germ-cells of 84 herself, for she had received no injections of lens since about three months before her mating with 53B1. In addition to this three-months interval must be added the time (about six months) it took her son 92C to come to sexual maturity. It is not impossible, however, that the defective condition of 92D was due to the placental transmission of antibodies, since, according to the investigations (in press, *Jour. of Inf. Dis.*) of our research assistant, George F. Forster, who made a special study of this point for us, antibodies (detectable precipitins) may linger on in the blood-stream of rabbits for upward of a year or more. Forster found, however, that in the fourteen rabbits he studied, the peak of antibody production occurred between ten and eleven days after the last injection of antigen. The antigen itself could be detected in the blood of the rabbits from seven and one-half to nine and one-half days.

As controls on the matings of female 84, in addition to matings of her sisters (85 and 86) to males 62 and 53B1, we have records of matings among her sibs, between her sibs and her offspring, and between her sibs and their own offspring. Although both females 85 and 86 were injected with pulped lens (tables 5 and 6) we got no eye defects in their offspring, even when bred to sons of 84. Thus, female 85 (sister of 84) was bred to male 62 February 14, 1920, and bore seven normal-eyed young; mated to 53B1 she bore six normal-eyed young; mated again to 62 she bore seven normal-eyed young; and finally, a mating between her and 92C, son of female 84, yielded a litter of six young with normal eyes. Likewise, female 86 (also a sister

of 84) mated to 62 bore three normal young in July, 1920; mated to 53B1 bore five young (104A series) with normal eyes, December 20, 1920; seven months later, by her own son (104A) she bore five normal-eyed young, and shortly after, by this same son she had a single young one with normal eyes; bred to 92 (son of 84), she bore seven normal-eyed young, October 20, 1921; and four months later by her brother, male 83, she had a litter of five, all normal-eyed. If there had been recessive eye defects in the strain to which female 84 belonged such inbreeding of close relatives should have brought them to light, but not a single defective-eyed individual occurred among these controls.

This new line, secured by injecting lens directly into the pregnant female, has also been crossed into a combination of the two older lines—the 3A1 and the 16A1—which were obtained by the use of fowl serum immunized against rabbit lens. The result of this interbreeding is shown at the right side of the chart in figure 1. A defective-eyed male, 71A3, of the 16A1 line, was mated to a normal-eyed female, 91A5, the father of which was of normal stock, the mother, from the 3A1 line. The defective-eyed daughter, 98B1, of the resulting litter was then bred to the defective-eyed son, 92B1, of the 'direct lens' (female 84) line. Every one of the resulting five young (121A series, has both eyes defective.

#### MALE LINE EXTRACTIONS

Although in our first study (20) we showed through several breedings of defective-eyed males to normal females of unrelated strains that the defect can be transmitted through males to  $F_2$  and subsequent generations, and that it is therefore truly inherited and not merely a matter of placental transmission of antibodies or kindred substances, it seemed desirable to test this by still further matings. The charts shown in figures 3 and 4 give additional corroborative evidence of the actual hereditary nature of the anomalies. Male 3A1 with defective left eye was mated to female 17 of a normal unrelated stock (fig. 3). The eight young (26A series) born of this mating all had normal eyes. As soon as she became of breeding age, female 26A7 was mated to her defective-eyed father, 3A1, and of the four

resulting young, one male, 32B, had a defective left eye. Female 26A6, mated to 28A4, a male with both eyes markedly defective, bore four young, of which two females, 46A1 and 46A2, each had a defective left eye. One of these females, 46A1, was next mated to the male 32B with defective left eye. The resulting litter of nine (fig. 3; 61A series) contained one male, 61A1, with both eyes defective, two females, 61A2 and 61A3, with both eyes defective, two other males and three other females with normal eyes, and one which died shortly after birth before the condition of the eyes could be determined.

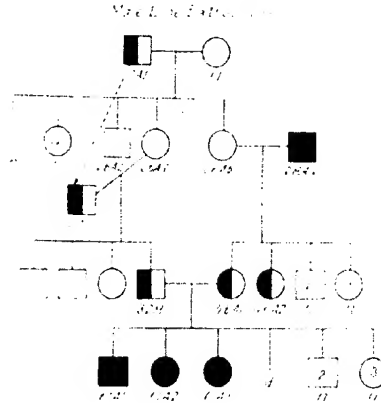


Fig. 3. Inheritance of the defects through the male line. It is plain that individuals of the 32B, 46A, and 61A series could have derived their defects only through male ancestry, since female 17 was of normal and unrelated stock. Symbols same as in figure 1.

The chart shown in figure 4 is even more interesting in that a defect from a male, 3A1, has been transferred into two different normal strains, and after having remained unexpressed through two generations has been brought to view again in the great granddescendants by breeding together individuals (90A2 and 91A4) of the respective strains. Incidentally the matings shown in this chart also answer the question as to whether only albino rabbits exhibit the defects. All of the rabbits used in

the original experiments were albinos but pigmentation has been introduced into the stock by means of a black male, number 25, figure 4. Female 97A1 (fig. 4), a granddaughter of this male was not only pigmented (black-and-white pattern) but showed the extracted defect in the left eye in the form of cleft iris, slightly opaque lens, and silvery hue. Male 97A2, likewise pigmented, showed defects in each eye similar to those in the left eye of the female. The daughter of this pair, 120A, bears a corresponding black-and-white color pattern and has a left eye somewhat reduced in size with silvery look and slightly opaque lens.

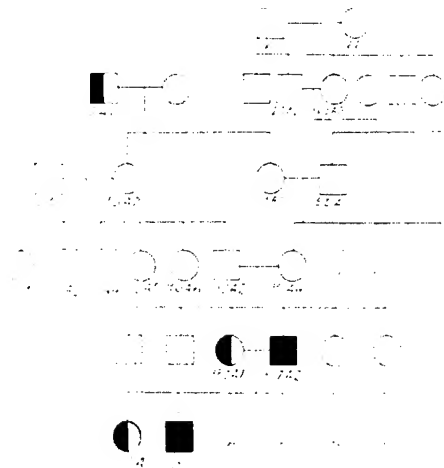


Fig. 4. Inheritance of the defects through the male line. Symbols same as in figure 1. Individuals 11, 2, and 22 were of normal, untreated stock, hence the defects seen in the 97A and 120A series could only have been derived from the albino male, 3A1. Pigmentation was introduced into the stock through male 25. The defective-eyed 97A1 and 97A2 are pigmented.

#### DISCUSSION AND CONCLUSIONS

We feel that in establishing and developing from unrelated stocks two more defective-eyed strains of rabbits—the one (16A1 line) by the use of fowl serum immunized to rabbit lens, the other (84 line) by direct injection of rabbit lens into a preg-

nant rabbit—we have placed our results beyond the bounds of coincidence or chance. If they are still not sufficiently convincing we can also cite the case of lens defects secured in the young of guinea-pigs by injection of rabbit lens into pregnant females, although we are not yet ready to report on this series of experiments.

The suggestion has been made to us that perhaps by inbreeding we have merely uncovered a recessive defect already present in the stock. In reply to this, as regards our first defective-eyed line (designated as the 3A1 line), we can only say that other litters from the same parents (female 1 and male 2) were secured and none of them nor their descendants, although bred as brother and sister matings, have shown the defects. For example, from matings of female 3B2 (daughter of female 1, by male 2) by her brother, 3B3, twelve young (C4A and B series) were secured, all normal eyed; and from brother and sister matings of the latter, of the thirty young secured not one had defective eyes. If the defect were one originally in the strain it is strange that it did not reveal itself in these collateral inbred lines. Moreover, even when 3A1, the first defective-eyed male secured, was bred back to his mother, the five resulting young were normal-eyed.

But even supposing that the improbable happened that for our experiments, by chance, we just happened to hit upon an individual which was carrying a recessive eye defect ready to be revealed in her descendants after treatment with serum immunized against rabbit lens, is it probable that the same defect would also happen by chance in our unrelated 16A1 line, and does this theory of chance not become wholly untenable when we attempt to carry it over to still a third unrelated line (84 line)? Attention, however, should perhaps be called to one fact. The three lines in question were all albinos. It is possible that the eyes of albinos are more easily affected than those of pigmented rabbits. However this may be, our records show that the defect can easily be crossed into pigmented stock, once it has been engendered (see fig. 4 in which 97A1 and 97A2 are pigmented rabbits). A further investigation of

this point is being undertaken by one of our associates, working with pedigreed lines of New Zealand Reds.

Perhaps the most interesting fact brought to light in the present study is possibility of directly or indirectly inducing germinal changes by means of antibodies engendered in an animal's own body against tissue taken from other individuals of its own species. Such a result, together with those obtained by the senior author (Guyer, '22 a) in the production of antibodies against an individual's own spermatozoa, lend support to the idea that an animal can build antibodies against its own tissues when these are misplaced, altered, or injured in some way, and that such antibodies may induce germinal changes. Since this point has been discussed in two recent papers (Guyer, '22 b, '22 c) it need not be entered into here.

As to the general nature of the eye defects, as seen in the living animals, little additional information over that given in our earlier ('20) study is forthcoming. The one outstanding defect that is nearly always present, no matter what the other anomalies may be, is some degree of opacity of the lens. But this is not invariable, as we have found a few instances in which the lens remains apparently clear although the rabbit may have other abnormalities in the eye. It should be stated, however, that we have recorded lenses or eyes as normal or abnormal on the basis of what our mere unaided macroscopic examination of the eyes revealed. Two experienced ophthalmologists, Doctors Davis and Neff, who are now making a careful and detailed examination of some of our material by means of the ophthalmoscope and by dissections, have pointed out that the lenses of some of our rabbits recorded as normal are really in some degree defective. This discrepancy may be due to an oversight on our part because of our less thorough examination of the eyes or to the fact that in our strains of treated stock a lens which appears normal in early life may become more or less cataractous later. We have knowledge of several cases in which the latter has happened, also one or two in which a lens, previously somewhat opaque, has cleared up. When, as pointed out in our earlier paper ('20), we take into account the method

of embryonic development of the eye, it is not unreasonable to infer that at least most of the other defects—reduced size, cleft iris, displaced lens, persistent hyaloid artery, etc.—are probably attributable to defects in the early lens. Ophthalmologists such as Dr. Lucien Howe of Buffalo, New York, and Doctors Davis and Neff of Madison, Wisconsin, who have studied the eyes in a number of our rabbits, tell us that the bluish or silvery appearance so common in many of the defective eyes is due to detachment of the retina instead of the milkiness of the lens, as we had originally supposed.

The following excerpts from our notebook show the conditions of the eyes in representative individuals of our defective-eyed stock as disclosed by autopsies performed by H. M. Smith:

3A1. Male, killed 4-21-22. Right eye: apparently normal although layers of retina were indistinct. Left eye: lens completely calcified and much misshapen; vitreous cavity filled with mass of opaque tissue.

4D1. Male, killed 3-24-22. Both eyes apparently normal in life; autopsy showed eyes almost normal, although the layers of the retina were indistinct and the cornea irregular in thickness and somewhat wrinkled.

6A1. Male, killed 3-25-22. Right eye: coloboma; lens large and spherical; conjunctiva thickened; cornea of irregular thickness; retina a disorganized mass of cells, mostly thin, but associated with a loose mass of connective tissue back of lens. Left eye: lens rolled backward in region of coloboma with an angular spur of the lens fitting into a pocket in the sclera; no retina and doubtful choroid in front of pocket; large mass of connective tissue back of lens, associated with disorganized retina; dorsally, retina has fairly typical structure.

6A3. Female, killed 6-3-22. Right eye: coloboma; small bulge near optic nerve; lens large and round; retina fairly typical dorsally, but disorganized posteriorly and absent ventrally. Left eye: normal except that cornea is irregular and retina shows some signs of degeneration.

10D2. Female, died. Head in formalin several weeks, enucleated 3-14-22. Right eye: coloboma; large posterior staphyloma; lens large and round; deep anterior cavity; lens rolled back in region of coloboma; cornea white and thick, with many nuclei; retinal tissue almost unrecognizable, absent ventrally; posterior portion of vitreous cavity filled with mass of connective and disorganized retinal tissues; lens lacks clear-cut structure of normal lens. Left eye: coloboma; eyeball with large posterior bulge; vitreous cavity filled with mass of tissue; lens large and round; cornea thick; sclera very thin at bulge;

posterior portion of ball a heavy network of connective tissue; retina scarcely recognizable and lacking ventrally.

28C1. Female, killed 9 1 22. Both eyes microphthalmic; corneas, and probably lenses, opaque; not yet sectioned.

28D2. Female, killed 9 1 22. Both eyes apparently normal; not yet sectioned.

48A. Female, killed 3 22 22. Right eye: coloboma and posteroventral bulge; cornea nearly normal, sclera very thin ventrally; retina practically structureless and absent ventrally. Left eye: coloboma and a posteroventral bulge.

57A4. Male, killed 3 14 22. Both eyes nearly normal; corneas rough; some evidence of retinal degeneration; lenses apparently normal.

71B2. Female, died 6 2-22; eyes fixed 4 hours later. Right eye: microphthalmia; lens opaque, round, rough in outline, structureless, and fills entire cavity; optic nerve prominent and surrounded by heavy cellular sheath; its fibers spread slightly and disappear in mass of connective and disorganized retinal tissues which obliterates vitreous cavity; cornea thick, narrow, heavily nucleated; iris with no pupil; anterior chamber small. Left eye: coloboma; bulge, involving optic nerve; ball smaller than normal; lens round, indented on one side, surrounded by a mass of connective tissue; cornea thick; optic nerve as in right; its fibers spread out somewhat but have no connection with retina, which occurs as a rather structureless mass continuous with mass of connective tissue noted above.

92A. Male, killed 8 4 22. Right eye: microphthalmia; lens displaced, small, calcified; ball very small and cavities almost obliterated. Left eye: normal size and shape. Lids were inflamed and unable to close. Cornea covered by dried exudate. Not sectioned.

92B1. Male, killed 9 21 22. Each eye: microphthalmia; very much distorted; lids and conjunctiva infected; cornea small and opaque.

97A1. Female, killed 11 3 22. Pigmented rabbit. Eyes not yet sectioned. Right eye: apparently normal. Left eye: coloboma involving optic nerve.

97A2. Male, killed 11 3 22. Pigmented rabbit. Eyes not yet sectioned. Right eye: coloboma involving region of optic nerve; slight bulging of optic nerve. Left eye: coloboma apparently confined to iris; sclera thickened along ventral side.

98A2. Female, killed 11 3 22. Each eye apparently normal; not yet sectioned.

121A1. Male, died 4 21 22. Right eye: coloboma; posteroventral bulge of eyeball; lens large and round; cornea normal; lens with secondary posterior growth region; retina and bulging area as in left except that bulge is more strictly confined to sclera; in the region of the coloboma both retina and choroid are missing. Left eye: coloboma; cornea rough; lens large, elongated in antero-posterior axis with secondary growth region on anterior surface; retina normal in places but for the most part indistinct and associated with connective tissue masses

in vitreous cavity; a large posterior bulge forms a separate cavity the walls of which contain scleral choroid, and possibly some nervous tissue.

Newborn young of 97A1 Female, and 97A2 Male, killed by mother 5-31-22.

- (a) Pigmented. Eyes apparently normal.
- (b) Pigmented. Coloboma in each eye, involving optic nerve; each rather atrophic (6.5 mm. width—normal 8 to 9 mm.).
- (c) White. Lenses calcified; eyes otherwise apparently normal.
- (d) Pigmented. One eye apparently normal, other as in (b).
- (e) Pigmented. Eyes apparently normal.

Newborn young of 121A2 Female, and 71B1 Male, all white; died, fixed within few hours 12-13-22.

- (a) Each eye small and with coloboma; right sclera bulged and somewhat thickened posteriorly.
- (b) Each apparently normal.

(c) Right eye: cornea somewhat opaque, no apparent coloboma, but pupil moved dorsally in compensation for a huge ventral staphyloma; by squeezing ball, position of iris can be changed, suggesting that iris is not attached to sclera; just behind conjunctiva a smaller staphyloma extends from dorsal to ventral side of ball.

Left eye: opaque band apparently across lens, dividing pupil into two clear areas; eye otherwise appears normal.

(d) Right eye: ball filled with blood so that structure is obscured; cut in enucleation, revealing a vitreous cavity filled with membrane and no distinguishable lens.

Left eye: dorsoventrally slit-shaped pupil; ball small; possibly coloboma.

(e) Right eye: may be normal but looks somewhat opaque. Left eye: white band as in left eye of (c); definite opaque spot on dorsal portion of cornea; eye small.

Young of 130A3 Female, and 71B1 Male; more than half grown. Died 12-13-22, frozen over night, fixed next morning. Right ball firmly attached in socket by connective tissue; coloboma; huge ventral staphyloma; multiple, involving entire ventral and posterior portions of ball. Left eye: microphthalmia; very small, surrounded by large mass of connective tissue; cornea opaque.

#### *Remarks*

1. Detached retina cannot be diagnosed in these preparations because normal retinas are frequently detached in process of preparation.

2. Defective eyes are histologically decidedly structureless when compared with normal eyes. This condition extends to all the tissues involved, but apparently less to choroid than others.

3. Defective lenses not infrequently tend to be rounded and larger than normal.

The following excerpts record some additional histological details of yet other defective-eyed individuals as reported by

Helen E. McDonald from studies made on eyes which had been sectioned in paraffin or celloidin:

6A4. Right eye normal. Left eye: small opaque; retina nodular, with indistinct layers, and lacking in some places; outer molecular layer, when recognizable, is of varying thickness.

10A3. Right eye: very small, lens granular, filling almost the entire cavity and practically obliterating the vitreous body; retina nodular, not arranged in layers; choroid much reduced. Left eye: of normal size and appearance, viewed externally; retina indistinct with layers fused.

45A5. Right eye: normal? Left eye: coloboma; opaque lens; microphthalmic; retina not organized into layers; sections into the optic nerve show no retina connected with it.

47A. Eyes apparently normal although more cones than rods were found in the retina; the normal ratio is three rods to one cone.

71A4. Right eye: retina shows a nodular outer nuclear layer; no layer of rods and cones, in places. Left eye: markedly small; marked staphyloma; retina asymmetrical with no definite layers.

71A6. Right eye: no discoverable retina. Left eye presumably normal.

91A4. Eyes apparently normal as seen from without. Right eye: retina nodular with converging outer and inner nuclear layers. Left eye: some nodules in retina, with rods and cones missing in places.

41B1. Right eye: nodular retina with rods and cones missing in places. Left eye: opaque; sunken; small; marked proliferation of sclerotic tissue; no retina.

28B. Right eye: smaller than normal; seems normal as regards retina and sclera. Left eye: staphyloma; rods and cones missing in places; outer nuclear layer is of varying thickness.

#### *Remarks*

1. The average thickness of the retina in the eyes of the defective-eyed individuals studied was less than the average thickness (0.14 mm.) of the retina in normal-eyed rabbits. A similar thinning of the choroid was observable.

2. With decrease in size of the eye, the lens diminishes relatively less in proportion, hence it may largely obliterate the vitreous body and occupy its place.

3. The presence of an optic nerve in connection with even the most defective eyes leads to the inference that those eyes which have no retina display degeneration rather than an inhibition of development, since the optic nerve is formed by the growth of fibers from the retina inward toward the brain along the path of the optic stalk.

The possibility that we have developed antibodies against other eye-tissues as well as against the lens should not be overlooked. No special precautions were taken to secure purely

lens-tissue when we removed the lenses for use in the original injections. Doubtless traces of both aqueous and vitreous humor were present in the final emulsion used for injection. And if proteins from other parts of the eye, as someone has suggested, are ever in solution or suspension in these humors, they too were present in the original antigen. Another possibility also exists. Eyes rendered defective primarily as the result of lens anomaly may secondarily have developed antibodies against their own tissues, when the latter have been forced into abnormal conditions. It is a noteworthy fact that later generations of the defective lines are characterized by more marked degeneration of the eyes.

As to the genetics of the defects, we have little to add to our earlier ('20) study. In general the defective-eyed condition behaves after the manner of a mendelian recessive, although we have never been able to isolate a pure defective-eyed strain which will not throw some normal-eyed offspring. We have come near to it, however, in some of our later matings, and it may be that individuals of the 121A and B series, or the 130A series (fig. 1) will give us such a result.

It is possible, indeed probable, judging from the different kinds of defects that may appear, that we are dealing with a number of unit factors, although as pointed out it is not impossible that all others are initiated by an early defective condition of the lens.

The question of the specificity of the lens reactions in our experiments is still before us. As pointed out in our earlier study ('20) knowing how susceptible in early embryogeny the eye is to any kind of deleterious influence, the general inclination of an embryologist, is to regard the result as due to a general poisonous or inhibitive effect rather than to specific antibodies in the blood serum. We can only say that we have never obtained the defects in question except with serum carrying specific antibodies. Since the experiments described in our 1920 paper were performed, we, together with others in our laboratory, have been doing many experiments in which shortly before or during pregnancy female rabbits have been injected with typhoid

fever vaccines or living typhoid germs, or with various kinds of foreign serum or serum immunized against proteins other than lens, and in not a single one of more than five hundred young born after such treatments has there been a case of eye defect. But even should the defect have originally been a general rather than a specific one, it is obvious that the germinal condition sooner or later must have become specific since the anomaly reappears generation after generation without any recognizable accompanying malformations of other parts of the body. In our opinion, however, it is not impossible, or improbable, that a fetal defect engendered in the eye or elsewhere by even general means might lead to the development of antibodies which could attack the germinal correlatives of the somatic part affected, and thereby inaugurate a specific germinal change.

On the point of placental penetration by antibodies, we have more evidence than was forthcoming at the time of publication of our earlier ('20) paper. Notwithstanding negative results, particularly on the pig and the kid, reported by several investigators recently (Reymann, '20) we are convinced that in rabbits, at least, antibodies penetrate the placenta. It is possible that the placentae of swine and ruminants, which are of the diffuse and the cotyledonary types respectively, differ in penetrability from the more intimate discoidal type possessed by the rabbit. However this may be, we feel sure of the conditions which exist in the rabbit. We have especially tested the matter as regards precipitins and agglutinins.

For the precipitin test pregnant rabbits were intravenously injected with sheep-serum. The rabbits were killed after a pregnancy of twenty-five days and the young were removed with the greatest care, each still surrounded by its amniotic fluid. Each fetus was washed twice in normal saline to prevent any possibility of contamination by the mother's blood. In all, seven pregnant rabbits were so tested, and in every case the precipitins were found in the blood of the fetuses. The experiments are given in detail in a paper in press.

In testing for placental penetration of agglutinins, five pregnant rabbits immunized to the bacillus of typhoid fever were used. The mothers and the fetuses were tested by the usual Widal reaction, and each gave positive reactions. Since the details are given at some length together with tabulations of the separate experiments in the paper mentioned in the preceding paragraph, they need not be repeated here.

The question has been raised occasionally by serologists as to whether our original modifications were cytolytic in nature or whether they should be attributed to some other type of serological reaction. In this connection we should like to call attention to our concluding remarks in our second paper ('20): ". . . still less are we inclined to undertake any categorical exposition of the serological detail. We are more interested in presenting the facts that our experiments reveal." In our first paper ('18) we gave some reasons for believing that the results indicated a true cytolytic effect, although we also pointed out the possibility that the clouding and opaquing so frequently observed in the lenses might be the result of a precipitin. From the careful researches of Hektoen ('18) fowl serum has been shown to be particularly good for the production of precipitins. On the other hand, although in its natural state it is somewhat cytolytic for rabbit corpuscles, *in vitro* at least, fowl serum is for some unknown reason inferior for the production of the type of cytolsins known as hemolysins. This fact which we ourselves, learned fairly early in our work has been pointed out by several workers, among them Takenouchi ('19) and Hyde ('22). But it does not necessarily follow that fowl serum is equally poor for the formation of other cytolsins.

In the face of all the facts—the liquefactions that sometimes are found, the reductions in size of the eye ball, the frequent resorptions which occur—we are still inclined to believe that the effect is partially if not largely cytolytic in nature. Since it can be induced apparently by antibodies developed through treating the pregnant mother directly with lens, the question of whether the fowl is or is not a good producer of cytolsins becomes, from our point of view, a fact of secondary importance.

We used it in our earlier experiments not because of any superior merit we found in it, but simply because it 'worked.'

A question that has frequently been asked us is whether our work does not 'prove' Lamarckism. We have made no such claim. We are not particularly interested in establishing or disestablishing any ism, but are concerned mainly in seeing what facts we can discover in the field of our researches. We feel that our experiments have shown that the germinal constitution can probably be altered by immunological influences, and have given our reasons for believing it not an untenable hypothesis that changes in an individual's tissues might engender such influences. In several papers of more general nature than our technical studies, the senior author (Guyer, '21, '22 a, '22 b, '22 c, '22 d) has expanded upon this theme, always, however, with the explicit statement that his remarks are to be regarded as interesting possibilities or working hypotheses rather than a record of established facts.

#### SUMMARY

Details are given concerning the production and inheritance of eye defects in two different strains of rabbits. In one strain the anomalies were induced in the fetal young by injecting into the mother a foreign serum immunized to rabbit lens; in the other strain similar defects were secured in the unborn young by direct injection of pulped lens into the pregnant mother. Pedigree charts of typical matings are included and discussed. More detailed descriptions of the various eye anomalies than those given in our earlier paper are provided. Reasons are advanced for believing the modifications to be a specific immunologic effect, and additional charts and descriptions of male line extractions showing that the hereditary nature of the defects is unquestionable, are presented. That germinal constitution can be altered by immunologic influences, the experimenters believe is the best working hypothesis to account for their results as they stand at present.

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## PLATE I

## EXPLANATION OF FIGURES

5. Female 73A2, showing buphthalmic eye with staphylomatous sclera. The eyeball is so rotated that the edge of the cornea is just visible at the upper outer angle of the lids; each eye has the lens opaque.
6. Female 139A2, conditions much as in figure 5.
7. An individual showing opaque lens, coloboma, and eyeball of reduced size.
8. Showing opaque lens and coloboma of the iris.





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